# EXHIBIT 2

**UNITED STATES DISTRICT COURT**
**DISTRICT OF NEW JERSEY**

| | |
|---|---|
| KING PHARMACEUTICALS, INC., KING PHARMACEUTICALS RESEARCH AND DEVELOPMENT, INC. and PHARMACEUTICAL IP HOLDING, INC.,<br><br>Plaintiffs,<br><br>v.<br><br>SANDOZ INC.,<br><br>Defendant. | Civil Action No. 08-5974 (GEB)(DEA) |

**EXPERT REPORT OF MICHAEL E. ELIA, M.D.**

I, Michael E. Elia, M.D., submit the following report on behalf of King Pharmaceuticals, Inc., King Pharmaceuticals Research and Development, Inc. (together, "King"), and Pharmaceutical IP Holding, Inc. ("Pharma IP") (collectively, "Plaintiffs") in this action.

## I. EXPERT QUALIFICATIONS

### A. Area of Expertise

1. I am an expert in the field of orthopaedic medicine, specializing in the evaluation and treatment of orthopaedic injuries. In particular, I have considerable expertise in the field of skeletal pain management.

### B. Educational Background

2. I received my Bachelor of Science degree in biology in 1978 from Boston College and my Medical Degree in 1982 from Georgetown University School of Medicine.

3. Following receipt of my Medical Degree, I did a General Surgery Internship at St. Elizabeth Hospital, in Boston, Massachusetts (1982-1983) and an Orthopaedic Residency at St. Luke's/Roosevelt Hospital Center in New York, New York (1983-1987).

**C. Relevant Professional Experience**

4. I am a licensed physician in New York State and am currently a physician and the Director in the Department of Orthopaedic Surgery at Lawrence Hospital, Bronxville, New York, and an Attending Orthopaedic Surgeon at St. Luke's/Roosevelt Hospital, New York, New York.

5. Over my career, I have been appointed to a number of positions including, Chairman of the Young Physicians Committee, Co-Chairman of the Membership Committee for the Westchester County Medical Society, the Lawrence Hospital Search Committee for Selecting Chief Executive Officer, and the Lawrence Hospital Surgical Planning Committee.

6. I am currently a Fellow in the American Academy of Orthopaedic Surgeons and sit on the Lawrence Hospital Medical Board. I am also a member of the New York State Medical Society, Westchester County Medical Society, and the Lawrence Hospital Physicians Organization. For additional information regarding my professional affiliations, please see my *curriculum vitae*, a copy of which is attached hereto as Exhibit A.

**D. Other Testimonial Experiences**

7. Within the last four years, I have provided sworn testimony in only one patent infringement case – Civil Action No. 04-5540 in the Eastern District of New York.

**E. Compensation**

8. I am being compensated at my usual rate of $500/hour in connection with this proceeding.

## II. BASES FOR OPINION

9. I have spent twenty years practicing as an orthopedic surgeon. In this time, I have treated hundreds of cases involving skeletal muscle pain. The opinions presented below are based upon my clinical experience, consideration of the following information and materials, and any additional information or material mentioned in the text of this report:

- United States Patent No. 7,122,566 ("the '566 patent") (attached as Exhibit B) and its prosecution history;
- The Skelaxin® package insert;
- Sandoz's metaxalone package insert (attached as Exhibit C);
- The Court's Markman Order and the Joint Claim Construction and Pre-Hearing Statement in this case; and
- The Court's memorandum opinion and order dated May 17, 2010.

## III. OVERVIEW OF OPINION

10. If called to testify in this action, I am prepared to testify that, in my opinion, claims 1-3, 5-12, and 14-22 of the '566 patent are infringed by Sandoz.

11. I am also prepared to serve in a teaching capacity concerning general practices of prescribing physicians.

## IV. RULE 26(A)(2)(B) DISCLOSURE REGARDING EXPERT TESTIMONY

### A. Background

12. King sells a metaxalone product under the trade name Skelaxin® that I prescribe in accordance with the information in its package insert. In my opinion, other doctors prescribe Skelaxin® in the same way. I understand that Sandoz is now selling a metaxalone product with a package insert that is virtually identical to the Skelaxin® package insert. The Sandoz metaxalone would likewise be prescribed in accordance with its package insert.

13. In my opinion, the information in Sandoz's package insert is critical for the safe and effective use of its metaxalone product. The package insert instructs patients, doctors and other medical care workers how to use the drug, how the drug works, and what potential risks are associated with use of the drug. The package insert also makes patients, doctors and other medical care workers aware of information concerning metaxalone's affect on cytochrome p450 enzymes and potential cytochrome p450 drug interactions.

14. Enzyme interactions, including those for the cytochrome p450 enzyme group, can play a role in drug interactions. In my opinion, it is important for medical workers, including prescribing physicians, and for patients to be informed of the potential for drug interactions mediated by enzymes such as cytochrome p450 enzymes. This is especially true since the number of patients taking multiple drugs simultaneously is on the rise, something that I have noticed in my own practice. Accordingly, knowing that cytochrome p450 enzymes interact with particular drugs can help prescribing physicians, such as myself, make important decisions for patients. As such, when I write prescriptions for my patients, I regularly ask them what other medications they are taking. I take this information into account when prescribing drugs to patients and advise patients accordingly. In my opinion, this is a common practice in the medical industry.

**B. Infringement of the '566 Patent**

15. I understand that this action concerns Sandoz's infringement of the '566 patent. I understand that there is direct infringement if each and every element of a patent claim is present in the accused activity. I understand that there is induced infringement if someone directly infringes the patent claim and Sandoz intended those actions by the direct infringers. I also understand that there is contributory infringement if the product is especially made or adapted for use in the claimed methods in the absence of substantial non-infringing uses.

16. In my opinion, Sandoz directly infringes Claims 1-3, 5-12, and 14-22 of the '566 patent. Claims 1, 5, and 6 are independent and Claim 1 is representative.

**1. Direct Infringement of Independent Claim 1**

17. Claim 1 states:

> A method of using metaxalone for treating a patient's condition, comprising
>
> providing a patient with metaxalone; and
>
> informing the patient or a medical care worker that metaxalone affects activity of a cytochrome p450 isozyme, and that administration of metaxalone with a substance that affects activity of a cytochrome p450 isozyme can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both.

Ex. B, at col. 64, ll. 47-56. This claim requires a "providing" step as well as an "informing" step. In my opinion, Sandoz directly meets both requirements.

18. In my opinion, Sandoz meets the "providing" step because it provides patients with metaxalone, *i.e.*, Sandoz manufactures, compounds, gives, distributes and sells metaxalone for use by patients. Ex. C.

19. In my opinion, Sandoz also meets the "informing" step. Sandoz's package insert for its metaxalone product contains the following information in a section titled "Distribution, Metabolism and Excretion":

> Metaxalone is metabolized by the liver and excreted in the urine as unidentified metabolites. Hepatic Cytochrome P450 enzymes play a role in the metabolism of metaxalone. Specifically, CYP1A2, CYP2D6, CYP2E1, and CYP3A4 and, to a lesser extent, CYP2C8, CYP2C9, and CYP2C19 appear to metabolize metaxalone.
>
> Metaxalone does not significantly inhibit major CYP enzymes such as CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Metaxalone does not significantly induce major CYP enzymes such as CYP1A2, CYP2B6, and CYP3A4 *in vitro*.

Ex. C. Sandoz's package insert informs doctors, such as myself, as well as other medical care workers or patients that metaxalone is metabolized by cytochrome p450 enzymes and, to a lesser degree, metaxalone inhibits certain cytochrome p450 enzymes and induces certain cytochrome p450 enzymes. Thus, Sandoz's package insert informs doctors, such as myself, other medical care workers or patients that metaxalone affects the activity of cytochrome p450 enzymes.

20. Based on this information, doctors, such as myself, other medical care workers and patients are also made aware that when metaxalone is administered with another substance, the plasma concentration, safety, and/or efficacy of one or both can be potentially affected.

21. More specifically, Sandoz's package insert further advises that:

> Metaxalone tablets may enhance the effects of alcohol and other CNS depressants.

Ex. C; "Warnings." Similarly, the "Information for Patients" section under the "Precautions" section of the package insert advises that:

> Metaxalone tablets may impair mental and/or physical abilities required for performance of hazardous tasks, such as operating machinery or driving a motor vehicle, especially when used with alcohol or other CNS depressants.

Ex. C. Moreover, the "Drug Interactions" section of the package insert also advises:

> The sedative effects of metaxalone and other CNS depressants (e.g., alcohol, benzodiazepines, opioids, tricyclic antidepressants) may be additive. Therefore, caution should be exercised with patients who take more than one of these CNS depressants simultaneously.

*Id.* In my opinion, these sections inform doctors or other medical care workers and patients that administering or dispensing metaxalone with substances such as alcohol, benzodiazepines, opioids and tricyclic antidepressants can affect the safety, efficacy and/or plasma concentration of metaxalone and/or these other substances.

22. Like metaxalone, alcohol and many benzodiazapines, opioids, and tricyclic antidepressants are also known to affect activity of cytochrome p450 enzymes as substrates, inducers or inhibitors of certain cytochrome p450 enzymes. Accordingly, Sandoz's package insert informs doctors, such as myself, as well as other medical care workers or patients that administration or dispensing of metaxalone with a substance that affects activity of a cytochrome p450 isozyme can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both.

23. Accordingly, in my opinion, Sandoz's package insert informs patients, physicians and other healthcare workers that administration of metaxalone with another substance mediated by p450 enzymes, such as alcohol, benzodiazepines, opioids and tricyclic antidepressants, can affect safety, plasma concentration and efficacy of both metaxalone and the other p450 enzyme mediated substance. Therefore, in my opinion, Sandoz infringes Claim 1 since it performs the "providing" and "informing" steps required by the claim.

24. Moreover, through additional literature, including its other package inserts, Sandoz also informs doctors, such as myself, other medical care workers and patients that other drugs, including drugs in the benzodiazapene, opioid, and tricyclic antidepressant drug families, act as substrates, inducers, or inhibitors of cytochrome p450 enzymes. For example, fluvoxamine, an antidepressant drug that is also a CNS depressant contains information concerning its metabolism and inhibition of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. D, Sandoz Fluvoxamine Label. Sandoz's alprazolam is a benzodiazepine drug and its package insert contains information concerning its metabolism, inhibition and inducement of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. E, Sandoz Alprazolam Label. Sandoz's amiodarone package insert contains

information concerning its metabolism, inhibition and inducement of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. F, Sandoz Amiodarone Label. Sandoz's bicalutamide label contains information concerning its inhibition of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. G, Sandoz Bicalutamide Label. Sandoz's bupropion package insert contains information concerning its metabolism of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. H, Sandoz Bupropion Label. Sandoz's fentanyl is an opioid and its package insert contains information concerning its metabolism, inhibition and inducement of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. I, Sandoz Fentanyl Label. Sandoz's Clomipramine is a tricyclic antidepressant and its package insert contains information concerning its metabolism of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. J, Sandoz Clomipramine HCL Label. Sandoz's perphenazine contains information concerning its metabolism and inhibition of certain cytochrome p450 enzymes and the potential for drug-drug interactions. Ex. K, Sandoz Perphenazine Label. Thus, Sandoz also explicitly informs patients and medical care workers concerning other substances' affects on cytochrome p450 activities.

**2. Direct Infringement of Independent Claim 5**

25. Claim 5 states:

> A method of using metaxalone to treat a patient's condition, comprising:
>
> providing a patient with metaxalone; and
>
> informing the patient or a medical care worker that a cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19 and that administration of metaxalone and a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both.

Ex. B, at col. 64, l. 65 to col. 65, l. 7.

26. Claim 5 differs from Claim 1 in that the "informing" step requires informing a patient or medical care worker that a cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19. Claim 5 further requires that the other substance *is* a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19.

27. As seen above, Sandoz's metaxalone package insert informs doctors, such as myself, other medical care workers and patients that metaxalone is metabolized by CYP1A2 and CYP2C19. Ex. C. Thus, for this reason and the reasons already explained above, in my opinion, Sandoz informs patients and medical care workers that a cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19 and that administration of metaxalone and a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both. Therefore, in my opinion, Sandoz infringes Claim 5 since it performs the "providing" and "informing" steps required by the claim.

### 3. Direct Infringement of Independent Claim 6

28. Claim 6 states:

> A method of using metaxalone to treat a patient's condition, comprising:
>
> providing a patient with metaxalone; and
>
> informing the patient or a medical care worker that metaxalone is an inhibitor, inducer, or substrate of a cytochrome p450 isozyme and administration of metaxalone with a substance that is an inhibitor, inducer, or substrate of the cytochrome p450 isozyme can affect the plasma concentration, safety or efficacy of the substance.

Ex. B, at col. 65, ll. 8-17.

29. Claim 6 differs from Claim 1 in that the "informing" step requires informing a patient or medical care worker that metaxalone is an inhibitor, inducer, or substrate of a cytochrome p450 isozyme. Claim 6 also requires that the other substance *is* an inhibitor, inducer, or substrate of a cytochrome p450 isozyme. For the reasons Sandoz infringes Claim 1 as discussed above, in my opinion, Sandoz informs patients and medical care workers that metaxalone is an inhibitor, inducer, or substrate of a cytochrome p450 isozyme and that administration of metaxalone and a substance that is an inhibitor, inducer, or substrate of a cytochrome p450 isozyme can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both. Therefore, in my opinion, Sandoz infringes Claim 6 since it performs the "providing" and "informing" steps required by the claim.

**4. Direct Infringement of Dependent Claims 2 and 8**

30. Claim 2 depends from Claim 1. Claim 8 depends from Claim 6. These claims further require that the substance that affects activity of a cytochrome p450 isozyme is an active agent with a narrow therapeutic index. As discussed above, Sandoz meets each element of independent Claim 1 and Claim 6.

31. As also discussed above, Sandoz's package insert specifically refers to benzodiazepines, opioids, and tricyclic antidepressants. Ex. C. Opioids and tricyclic antidepressants are examples of drug products that have narrow therapeutic indexes. Thus, Sandoz's package insert identifies substances that are metabolized by p450 enzymes and have a narrow therapeutic index. Accordingly, in my opinion, Sandoz directly infringes Claim 2 and Claim 8.

**5. Direct Infringement of Dependent Claims 3, 7 and 9-12**

32. Claim 3 depends from Claim 2 and further requires that the substance that affects activity of a cytochrome p450 isozyme is a substrate of CYP1A2, CYP3A4, CYP2B6, CYP2C19, CYP2D6, CYP2E1, or CYP2C9.

33. Claim 7 depends on Claim 6 and further requires that the cytochrome p450 isozyme is CYP1A2, CYP3A4, CYP2B6, CYP2C19, CYP2D6, CYP2E1, or CYP2C9.

34. Claims 9-12 depend from Claim 8. Claim 9 depends further requiring that the substance that is an inhibitor, inducer or substrate of the cytochrome p450 isozyme is a substrate of CYP1A2, CYP3A4, CYP2B6, CYP2C19, CYP2D6, CYP2E1, or CYP2C9. Claim 10 further requires that the active ingredient with the narrow therapeutic index is an inhibitor of the cytochrome p450 isozyme. Claim 11 further requires that the active ingredient with the narrow therapeutic index is an inducer of the cytochrome p450 isozyme. Claim 12 further requires that the active ingredient with the narrow therapeutic index is a substrate of the cytochrome p450 isozyme.

35. As discussed above, Sandoz infringes Claims 2, 6, and 8. For the same reasons already discussed above, Sandoz's package insert also identifies alcohol, benzodiazepines, opioids and tricyclic antidepressants which act as substrates, inhibitors, and inducers of the cytochrome p450 enzymes specified in the claims. Moreover, as stated above, tricyclic antidepressants and opioids have a narrow therapeutic index and act as inhibitors, inducers, and/or substrates of cytochrome p450 enzymes. Accordingly, in my opinion, Sandoz directly infringes Claims 3, 7, and 9-12.

**6. Direct Infringement of Dependent Claims 14-19**

36. Claims 14, 16, and 18 depend from Claim 1. Claim 14 further requires that metaxalone is an inducer of the p450 isozyme. Claim 16 further requires that metaxalone is an

inhibitor of the p450 isozyme. Claim 18 further requires that metaxalone is a substrate of the p450 isozyme.

37. Claim 15 depends from Claim 14 and further requires that the cytochrome p450 enzyme that is induced is CYP1A2 and CYP3A4. Claim 17 depends from Claim 16 and further requires that the cytochrome p450 isozyme that is inhibited is CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP2C9, CYP2E1, or CYP3A4. Claim 19 depends from Claim 18 and further requires that the cytochrome p450 isozyme is CYP1A2 or CYP2C19.

38. As discussed above, Sandoz infringes independent Claim 1. For the reasons discussed above, Sandoz's package insert informs doctors such as myself, other medical care workers and patients that metaxalone is metabolized by certain cytochrome P450 enzymes and to a lesser degree is an inducer or inhibitor of certain cytochrome P450 enzymes. As discussed above, Sandoz's package insert also identifies alcohol, benzodiazepines, opioids and tricyclic antidepressants which act as substrates, inhibitors, and inducers of the cytochrome p450 enzymes specified in the claims. Accordingly, in my opinion, Sandoz directly infringes Claims 14-19.

**7. Direct Infringement of Dependent Claims 20-22**

39. Claims 20-22 depend from Claim 1. Claim 20 further requires that the patient provided with metaxalone is a human. Claim 21 further requires that the patient provided with metaxalone has a musculoskeletal condition. Claim 22 further requires that the patient provided with metaxalone is receiving metaxalone therapy. As discussed above, Sandoz infringes Claim 1. In addition Sandoz's package insert states that Sandoz's generic product is intended for use in humans. Ex. C. Sandoz's package insert further states that its generic product is "indicated as an adjunct to rest, physical therapy and other measures for the relief of discomforts associated with acute, painful musculoskeletal conditions." *Id.* In addition, Sandoz's package insert

recommends ongoing, daily administration of Sandoz's generic product. *Id.* Thus, Sandoz directly infringes Claims 20-22.

**C. Induced and Contributory Infringement of the Claims**

40. In my opinion, Sandoz also encourages doctors and other medical care workers to practice the claimed methods. Doctors, pharmacists and other medical care workers will provide Sandoz's metaxalone to patients. The doctors, pharmacists and other medical care workers will also give Sandoz's package insert for its metaxalone product to at least some patients. Those patients will be informed by the contents of the package insert discussed above. Accordingly, in my opinion, Sandoz encourages patients, doctors and other medical care workers to use Sandoz's product in a way that practices the claimed methods.

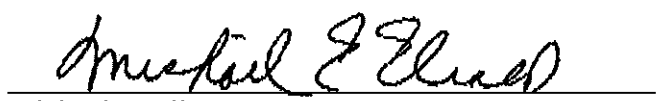
41. Sandoz's package inserts are publicly available and intended to reach doctors, other medical care workers and patients. In my opinion, Sandoz's package inserts educate and instruct doctors, other medical care workers and patients on how to use its products, and Sandoz therefore intends that doctors, medical care workers and patients use its products according to those package inserts. Specifically, Sandoz's intent to encourage doctors, other medical care workers, and patients to use Sandoz's product according to its package insert is manifested by the specific information and instructions contained in Sandoz's package inserts. Indeed, Sandoz's generic metaxalone product is packaged in a container with a label that specifically instructs the doctors, other medical care workers and patients to see the accompanying prescribing information that is in Sandoz's package insert. Ex. L. As discussed above, that package insert contains information and instructions that will cause doctors, other medical care workers, and/or patients to practice the methods claimed in Claims 1-3, 5-12 and 14-22. Accordingly, in my opinion, Sandoz instructs and encourages doctors, other medical care workers and/or patients to practice the methods claimed in Claims 1-3, 5-12 and 14-22.

42. In my opinion, Sandoz's metaxalone product is also especially adapted for use in the claimed methods and there are no substantial non-infringing uses. Indeed, also for the reasons above, Sandoz's metaxalone product is not just especially adapted for the claimed methods, it is solely adapted for those claimed methods. Given that FDA has not approved any other package insert that could be considered non-infringing, in my opinion, Sandoz's metaxalone product is not suitable for any substantial non-infringing use.

43. Consequently, by distributing, marketing and selling its metaxalone product, Sandoz induces infringement and contributorily infringes Claims 1-3, 5-12 and 14-22 of the '566 patent.

44. I reserve the right to supplement this report after reviewing deposition transcripts and documents that are made available to King after the date of execution of this report, or after reviewing any report made by any of Sandoz's expert witnesses in this case as well as any supporting materials included with those reports. I also reserve the right to disclose the identity and contents of any such additional materials that I may rely on in connection with, or in support of, my opinions expressed in this case.

Date: May 27, 2010



Michael E. Elia, M.D.

# EXHIBIT A

# MICHAEL E. ELIA, M.D.

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**SPOUSE:** Ellen M Elia Married August 21, 1982

**CHILDREN:** Christopher, Matthew, Michael, and Andrew

**EDUCATION:** Boston College B.S. Biology May, 1978 Magna Cum Laude
Georgetown University School of Medicine M.D. May, 1982

**POST GRADUATE TRAINING:**

General Surgery Internship — St. Elizabeth Hospital, Boston, MA, 6/82 - 6/83

Orthopaedic Residency — St. Luke's/Roosevelt Hospital Center, New York, NY, 7/83 - 6/87

**LICENSURE:** New York State License # 160233

**CERTIFICATION:**

Diplomate, American Board of Orthopaedic Surgery
Diplomate, National Board of Medical Examiners

**PROFESSIONAL ORGANIZATIONS:**

Fellow, American Academy of Orthopaedic Surgeons
New York State Medical Society
Westchester County Medical Society
Lawrence Hospital Physicians Organization, Secretary/Treasurer
Lawrence Hospital Medical Board 1992 - Present

**APPOINTMENTS:**

Chairman, Young Physicians Committee
Westchester County Medical Society
Lawrence Hospital Search Committee for Selecting Chief Executive Officer
Co-Chairman - Membership Committee - Westchester County Medical Society
Lawrence Hospital Surgical Planning Committee

**PUBLICATIONS:**

Occiput - C2 Posterior Cervical Fusion By the Onlay Technique
Authors: Michael B. Elia, MD, James Mazzara, MD, J. William Fielding, MD
Clinical Orthopaedics and Related Research, 1989

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# EXHIBIT B

US007122566B1

(12) **United States Patent**
**Du et al.**

(10) **Patent No.: US 7,122,566 B1**
(45) **Date of Patent: Oct. 17, 2006**

(54) **METAXALONE PRODUCTS, METHOD OF MANUFACTURE, AND METHOD OF USE**

(75) Inventors: **Jie Du**, Lansdale, PA (US); **Richard H. Roberts**, Lakewood, NJ (US)

(73) Assignee: **Mutual Pharmaceutical Company, Inc.**, Philadelphia, PA (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **11/364,468**

(22) Filed: **Feb. 28, 2006**

**Related U.S. Application Data**

(60) Provisional application No. 60/726,861, filed on Oct. 14, 2005.

(51) **Int. Cl.**
***A61K 31/42*** (2006.01)

(52) **U.S. Cl.** ..... **514/376**; 514/374

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

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Seidler, J, et al., "Identification and Prediction of Promiscuous Aggregating Inhibitors Among Known Drugs", Journal of Medicinal Chemistry, 46 (21):, 4477-4486 (2003).

Toth, PP, and Urtis J, "Commonly Used Muscle Relaxant Therapies for Acute Low Back Pain: A Review Carisoprodol, Cyclobenzaprine Hydrochloride, and Metaxalone," Clinical Therapeutics, 26(9): 1355-1367 (2004).

Prescribing Information for Skelaxin (metaxalone) as accessed at www.kingpharm.com on Dec. 21, 2005.

*Primary Examiner*—Dwayne Jones
(74) *Attorney, Agent, or Firm*—Cantor Colburn LLP

(57) **ABSTRACT**

Disclosed herein is a method of using metaxalone. In one embodiment, the method comprises obtaining metaxalone from a container providing information that metaxalone affects the activity of a cytochrome p450 isozyme. In another embodiment, the method comprises informing a user that metaxalone affects the activity of a cytochrome p450 isozyme. Also included are articles of manufacture comprising a container containing a dosage form of metaxalone, wherein the container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme. Also disclosed are a method of treatment and a method of manufacturing a metaxalone product.

**22 Claims, No Drawings**

# METAXALONE PRODUCTS, METHOD OF MANUFACTURE, AND METHOD OF USE

## CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. application Ser. No. 11/349,534 titled METAXALONE PRODUCTS, METHOD OF MANUFACTURE, AND METHOD OF USE, which was filed with Express Mail Label No. EV519660105US on Feb. 6, 2006, which claim the benefit of U.S. Provisional Application Ser. No. 60/726,861 filed Oct. 14, 2005, which are both hereby incorporated by reference in their entirety.

## BACKGROUND

This application relates to metaxalone products for therapeutic purposes, and in particular to improved methods of use of metaxalone.

Metaxalone, 5-[(3,5-dimethylphenoxy)methyl]-2-oxazolidinone, is used as a skeletal muscle relaxant. The mechanism of action of metaxalone in humans has not been established but may be due to general central nervous system depression.

Metaxalone was approved by the U.S. Food and Drug Administration (FDA) in 1962 as an adjunct to rest, physical therapy, and other measures for the relief of discomforts associated with acute, painful musculoskeletal conditions, such as muscles in spasm. Metaxalone is marketed in the United States under the brand name SKELAXIN®. The dosage forms currently approved for marketing are tablets containing 400 milligrams (mg) or 800 mg of metaxalone. The currently recommended dose for adults and children over 12 years of age is 800 mg, three to four times a day.

Food can affect gastric emptying, and may also alter the release of an active agent from a dosage form, the solubilization of the active agent, and the transport of the active agent across the intestinal wall. For lipophilic, water-insoluble active agents, fatty meals can increase gastric residence time thereby increasing the time available for solubilization and also may enhance the solubilization of the active agent by the lipids contained in the meal. According to U.S. Pat. No. 6,407,128, evaluation of the effect of food on the pharmacokinetics of metaxalone showed that food increased the rate and extent of absorption of a 400 mg oral dosage form in humans.

Studies directed to possible interactions of metaxalone with other active agents have been limited. There have been no detailed studies of the specific enzymes involved in metabolism of metaxalone or of the inhibitory or inducing effects of metaxalone on any Phase I or Phase II metabolic enzymes. In particular, there appear to be no published studies of the inhibitory and inducing effects of metaxalone on particular human cytochrome p450 isozymes or the possible metabolism of metaxalone by particular human cytochrome p450 isozymes.

Several major enzymes and pathways are involved in drug metabolism. Pathways of drug biotransformation are usually divided into two major groups of reactions: Phase I and Phase II metabolism.

Some typical examples of Phase I metabolism include oxidation, hydrolysis and reduction. Examples of Phase I enzymes involved in oxidation reactions are the cytochrome p450 monooxygenase system, the flavin-containing monooxygenase system, alcohol dehydrogenase and aldehyde dehydrogenase, monoamine oxidase, and peroxidases



for co-oxidation. Examples of Phase I enzymes involved in reduction are NADPH-cytochrome p450 reductase and reduced (ferrous) cytochrome p450. Examples of Phase I hydrolysis enzymes are epoxide hydrolase, esterases and amidases.

Phase II metabolism involves conjugation reactions. Typical conjugation reactions are glucuronidation, sulfation, amino acid conjugation, acetylation, methylation, and mercapturic acid conjugation. Examples of Phase II metabolic enzymes are glutathione S-transferases (GSTs), mercapturic acid biosynthetic enzymes (transpeptidases, peptidases, and N-acetylases), UDP-glucoron(os)yltransferases, N-acetyltransferases, amino acid N-acyl transferases, and sulfotransferases.

One of the most important groups of Phase I enzymes are the cytochrome p450 monooxygenase system enzymes. The cytochrome p450 enzymes are a highly diverse superfamily of enzymes. NADPH is required as a coenzyme and oxygen is used as a substrate. Each enzyme is termed an isoform or isozyme since each derives from a different gene.

Many members of the cytochrome p450 family are known to metabolize active agents in humans. Active agent interactions associated with metabolism by cytochrome p450 isoforms generally result from enzyme inhibition or enzyme induction. Enzyme inhibition often involves competition between two active agents for the substrate binding site of the enzyme, although other mechanisms for inhibition exist. Enzyme induction occurs when an active agent activates an enzyme or stimulates the synthesis of more enzyme protein, enhancing the enzyme's metabolizing capacity.

Cytochrome p450 isozymes identified as important in active agent metabolism are CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Examples of cytochrome p450 enzymes known to be involved in active agent interactions are the CYP3A subfamily, which is involved in many clinically significant active agent interactions, including those involving nonsedating antihistamines and cisapride, and CYP2D6, which is responsible for the metabolism of many psychotherapeutic agents, such as thioridazine. CYP3A4 and CYP1A2 enzymes are involved in active agent interactions involving theophylline. CYP2C9, CYP1A2, and CYP2C19 are involved in active agent interactions involving warfarin. Phenytoin and fosphenytoin are metabolized by CYP1A2, CYP2C9, CYP2C19, and CYP3A4; mexiletine is metabolized by CYP2D6 and CYP1A2; and propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2.

Additionally, several cytochrome p450 isozymes are known to be genetically polymorphic, leading to altered substrate metabolizing ability in some individuals. Allelic variants of CYP2D6 are the best characterized, with many resulting in an enzyme with reduced, or no, catalytic activity. Gene duplication also occurs. As a result, four phenotypic subpopulations of metabolizers of CYP2D6 substrates exist: poor (PM), intermediate (IM), extensive (EM), and ultrarapid (UM). The genetic polymorphisms vary depending on the population in question. For example, Caucasian populations contain a large percentage of individuals who are poor metabolizers, due to a deficiency in CYP2D6—perhaps 5–10% of the population, while only 1–2% of Asians are PMs. CYP2C9, which catalyzes the metabolism of a number of commonly used active agents, including that of warfarin and phenytoin, is also polymorphic. The two most common CYP2C9 allelic variants have reduced activity (5–12%) compared to the wild-type enzyme. Genetic polymorphism also occurs in CYP2C19, for which at least 8 allelic variants have been identified that result in catalyti-

cally inactive protein. About 3% of Caucasians are poor metabolizers of active agents metabolized by CYP2C19, while 13–23% of Asians are poor metabolizers of active agents metabolized by CYP2C19.

By understanding the unique functions and characteristics of Phase I and Phase II metabolic enzymes, physicians may better anticipate and manage active agent interactions and may predict or explain an individual's response to a particular therapeutic regimen.

There accordingly remains a need in the art for improved methods for the administration and use of metaxalone, in particular methods that take into account the effects of metaxalone on activity of Phase I and Phase II metabolic enzymes, including the cytochrome P450 isozymes.

## SUMMARY

Disclosed herein are methods of using metaxalone. Metaxalone can be used in the treatment of various diseases or conditions, including, for example, musculoskeletal conditions and head pain.

In one embodiment, the method comprises informing a user that metaxalone affects the activity of a cytochrome p450 isozyme.

In another embodiment, the method comprises informing a user that metaxalone is metabolized by a cytochrome p450 isozyme.

In another embodiment, the method comprises informing a user that metaxalone inhibits a cytochrome p450 isozyme.

In yet another embodiment, the method comprises informing a user that metaxalone induces a cytochrome p450 isozyme.

In another embodiment, the method comprises informing a user that metaxalone affects activity of a cytochrome p450 isozyme and that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance.

In another embodiment, the method comprises informing a user that metaxalone is metabolized by CYP1A2 or CYP3A4; and that there is a potential active agent interaction for metaxalone with an active agent that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that taking metaxalone with the active agent can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the active agent.

In another embodiment, the method comprises informing a user that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme and administration of metaxalone with a substance that is a substrate of the cytochrome p450 isozyme can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance.

In yet another embodiment, the method comprises obtaining metaxalone from a container providing information that metaxalone affects activity of a cytochrome p450 isozyme.

In yet another embodiment, the method comprises obtaining metaxalone from a container providing information that metaxalone is metabolized by a cytochrome p450 isozyme.

In yet another embodiment, the method comprises obtaining metaxalone from a container providing information that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme.

In yet another embodiment, the method comprises administering to a patient metaxalone and an active agent; and informing the patient that metaxalone affects activity of a cytochrome p450 isozyme.

In yet another embodiment, the method comprises informing a user that metaxalone affects activity of a cytochrome p450 isozyme; that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance can vary with administration of metaxalone with or without food.

In another embodiment, the method comprises obtaining metaxalone from a container providing information that metaxalone affects activity of a cytochrome p450 isozyme; that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance can vary with administration of metaxalone with or without food.

Also disclosed herein are methods of manufacturing a metaxalone product.

In one embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone affects activity of a cytochrome p450 isozyme.

In another embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone is metabolized by a cytochrome p450 isozyme.

In another embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme.

In yet another embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone affects activity of a cytochrome p450 isozyme; that administration of metaxalone with a substance can affect the plasma concentration, safety, or efficacy of the metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance can vary with administration of metaxalone with or without food.

Also disclosed herein are articles of manufacture comprising a container containing a dosage form of metaxalone.

In one embodiment, the container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme.

In another embodiment, the container is associated with published material informing that metaxalone is metabolized by a cytochrome p450 isozyme.

In another embodiment, the container is associated with published material informing that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme.

In yet another embodiment, the container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme; that administration to a patient of metaxalone with a substance can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxa-

lone or the substance can vary with administration of metaxalone with or without food.

In yet another embodiment, the article comprises a container comprising a dosage form of metaxalone, and published material. In one embodiment, the published material informs that there is a potential active agent interaction with warfarin; or that administration with warfarin can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or warfarin. In another embodiment, the published material informs that metaxalone is a substrate of CYP1A2 or CYP2C19, or that metaxalone is an inhibitor of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4, or that metaxalone is an inducer of CYP1A2 or CYP3A4.

Also disclosed herein is an article of manufacture comprising packaging material and a product contained within the packaging material, wherein the product comprises, as at least one active ingredient, metaxalone, and wherein the packaging material comprises a label approved by a regulatory agency for the product which states that metaxalone affects activity of a cytochrome p450 isozyme.

Also disclosed herein is a method of using an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or a substrate of a cytochrome p450 isozyme.

In one embodiment, the method comprises informing a user that metaxalone affects activity of a cytochrome p450 isozyme and that administration of the active agent with metaxalone can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or metaxalone.

In another embodiment, the method comprises obtaining an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or a substrate of a cytochrome p450 isozyme from a container providing information that metaxalone affects activity of a cytochrome p450 isozyme and that administration of the active agent with metaxalone can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or metaxalone.

Also disclosed herein is a method of manufacturing a pharmaceutical product comprising an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or a substrate of a cytochrome p450 isozyme.

In one embodiment, the method comprises packaging a dosage form of the active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or a substrate of a cytochrome p450 isozyme with information that metaxalone affects activity of a cytochrome p450 isozyme and that administration of the active agent with metaxalone can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or metaxalone.

Also disclosed herein is an article of manufacture comprising a container containing a dosage form of an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or a substrate of a cytochrome p450 isozyme. The container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme and that administration to a patient of the active agent and metaxalone can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or metaxalone.

These and other embodiments, advantages and features of the present invention become clear when detailed description and examples are provided in subsequent sections.

## DETAILED DESCRIPTION

The terms "a" and "an" do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item. The term "or" means "and/or". The terms "comprising", "having", "including", and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to"). Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. The endpoints of all ranges are included within the range and independently combinable. All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention as used herein. Unless defined otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

An "active agent" means a compound (including metaxalone), element, or mixture that when administered to a patient, alone or in combination with another compound, element, or mixture, confers, directly or indirectly, a physiological effect on the patient. The indirect physiological effect may occur via a metabolite or other indirect mechanism. When the active agent is a compound, then salts, solvates (including hydrates) of the free compound or salt, crystalline forms, non-crystalline forms, and any polymorphs of the compound are included. Compounds may contain one or more asymmetric elements such as stereogenic centers, stereogenic axes and the like, e.g., asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. These compounds can be, for example, racemates or optically active forms. For compounds with two or more asymmetric elements, these compounds can additionally be mixtures of diastereomers. For compounds having asymmetric centers, all optical isomers in pure form and mixtures thereof are encompassed. In addition, compounds with carbon—carbon double bonds may occur in Z- and E-forms, with all isomeric forms of the compounds. In these situations, the single enantiomers, i.e., optically active forms can be obtained by asymmetric synthesis, synthesis from optically pure precursors, or by resolution of the racemates. Resolution of the racemates can also be accomplished, for example, by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, using, for example a chiral HPLC column. All forms are contemplated herein regardless of the methods used to obtain them.

All forms (for example solvates, optical isomers, enantiomeric forms, polymorphs, free compound and salts of an active agent) of metaxalone or other active agent may be employed either alone or in combination.

"Active agent interaction" refers to a change in the metabolism of an active agent in a patient that can occur with co-administration of a second active agent. A "potential active agent interaction" refers to an active agent interaction between two active agents that is theoretically possible based on knowledge that one of the active agents is metabolized by a given cytochrome p450 isozyme and that the

second of the active agents is a substrate, inhibitor, or inducer of that cytochrome p450 isozyme.

"Administering metaxalone with a substance" means metaxalone and the substance are administered simultaneously in a single dosage form, administered concomitantly in separate dosage forms, or administered in separate dosage forms separated by some amount of time that is within the time in which both metaxalone and the substance are within the blood stream of a patient. The metaxalone and the substance need not be prescribed for a patient by the same medical care worker. The substance need not require a prescription. Administration of metaxalone or the substance can occur via any appropriate route, for example, oral tablets, oral capsules, oral liquids, inhalation, injection, suppositories or topical contact.

"Affects" include an increase or decrease in degree, level, or intensity; a change in time of onset or duration; a change in type, kind, or effect, or a combination comprising at least one of the foregoing.

As used herein, "allelic variant" means one of the alternative forms at a genetic locus on a single chromosome. For loci in most of the human genome, a human has two chromosomes, which may carry the same or two different allelic variants.

"Altering the dose of an active agent" can mean tapering off, reducing or increasing the dose of the active agent, ceasing to administer the active agent to the patient, or substituting a second active agent for the active agent.

"Bioavailability" means the extent or rate at which an active agent is absorbed into a living system or is made available at the site of physiological activity. For active agents that are intended to be absorbed into the bloodstream, bioavailability data for a given formulation may provide an estimate of the relative fraction of the administered dose that is absorbed into the systemic circulation. "Bioavailability" can be characterized by one or more pharmacokinetic parameters.

A "dosage form" means a unit of administration of an active agent. Examples of dosage forms include tablets, capsules, injections, suspensions, liquids, emulsions, creams, ointments, suppositories, inhalable forms, transdermal forms, and the like.

The term "effective amount" or "therapeutically effective amount" means an amount effective, when administered to a patient, to provide any therapeutic benefit. A therapeutic benefit may be an amelioration of symptoms, e.g., an amount effective to decrease the symptoms of an acute musculoskeletal condition, such as muscle spasms. In certain circumstances a patient may not present symptoms of a condition for which the patient is being treated. A therapeutically effective amount of an active agent may also be an amount sufficient to provide a significant positive effect on any indicium of a disease, disorder, or condition, e.g. an amount sufficient to significantly reduce the frequency and severity of muscle spasms. A significant effect on an indicium of a disease, disorder, or condition is statistically significant in a standard parametric test of statistical significance, for example Student's T-test, where $p \leqq 0.05$. An "effective amount or "therapeutically effective amount" of metaxalone may also be an amount of about 3600 mg per day or less, about 3200 mg per day or less, about 50 mg to about 3600 mg per day, or of any dosage amount approved by a governmental authority such as the US FDA, for use in treatment. In some embodiments amounts of 3200 mg metaxalone per day, 800 mg metaxalone per unit dosage form, or 400 mg metaxalone or less per unit dosage form is an "effective amount" or "therapeutically effective amount" of metaxalone.

"Efficacy" means the ability of an active agent administered to a patient to produce a therapeutic effect in the patient.

As used herein "food" means a solid food with sufficient bulk and fat content that it is not rapidly dissolved and absorbed in the stomach. More specifically, the food is a meal, such as breakfast, lunch, or dinner. A dosage of metaxalone administered to a patient "with food" or in a "fed" state is administered to the patient between about 30 minutes prior to about 2 hours after eating a meal; more specifically, the dosage is administered within 15 minutes of eating a meal. The terms "without food" or "fasted" are defined to mean the condition of not having consumed solid food for about one hour prior to until about 2 hours after such consumption.

"Head pain" includes any painful conditions of the head, but particularly includes headaches, such as migraines, cluster headaches, tension headaches, or tension related migraines. Head pain further includes painful facial conditions such as TMJ (temporomandibular joint) disorders.

"Informing" means referring to or providing, published material, for example, providing an active agent with published material to a user; or presenting information orally, for example, by presentation at a seminar, conference, or other educational presentation, by conversation between a pharmaceutical sales representative and a medical care worker, or by conversation between a medical care worker and a patient; or demonstrating the intended information to a user for the purpose of comprehension.

As used herein, an enzyme "metabolizing" a substance means the enzyme can chemically transform the substance.

A "medical care worker" means a worker in the health care field who may need or utilize information regarding an active agent, including a dosage form thereof, including information on safety, efficacy, dosing, administration, or pharmacokinetics. Examples of medical workers include physicians, pharmacists, physician's assistants, nurses, aides, caretakers (which can include family members or guardians), emergency medical workers, and veterinarians.

As used herein, "metaxalone therapy" refers to medical treatment of a symptom, disorder, or condition by administration of metaxalone.

The term "musculoskeletal condition" includes any condition affecting the muscles, tendons, ligaments, bones, joints, and associated tissues that move the body and maintain its form. Such conditions include conditions that originate in the muscles, tendons, ligaments, or bones and associated tissues or conditions that originate elsewhere in the body, for example in the central or peripheral nervous system, that are manifested in the muscles, tendons, ligaments, bones, joints or associated tissues.

A substance having a "narrow therapeutic index" (NTI) means a substance falling within any definition of narrow therapeutic index as promulgated by the U.S. Food and Drug Administration or any successor agency thereof, for example, a substance having a less than 2-fold difference in median lethal dose (LD50) and median effective dose (BD50) values, or having a less than 2-fold difference in the minimum toxic concentration and minimum effective concentration in the blood.

"Oral dosage form" includes a dosage form for oral administration.

A "patient" means a human or non-human animal in need of medical treatment. Medical treatment can include treat-

ment of an existing condition, such as a disease or disorder, prophylactic or preventative treatment, or diagnostic treatment. In some embodiments the patient is a human patient.

A "pharmaceutical supplier" means a person (other than a medical care worker), business, charitable organization, governmental organization, or other entity involved in the transfer of active agent, including a dosage form thereof, between entities, for profit or not. Examples of pharmaceutical suppliers include pharmaceutical distributors, pharmacy chains, pharmacies (online or physical), hospitals, HMOs, supermarkets, the Veterans Administration, or foreign businesses or individuals importing active agent into the United States.

"Pharmacokinetic parameters" describe the in vivo characteristics of an active agent (or surrogate marker for the active agent) over time, such as plasma concentration (C), $C_{max}$, $C_n$, $C_{24}$, $T_{max}$, and AUC. "$C_{max}$" is the measured concentration of the active agent in the plasma at the point of maximum concentration. "$C_n$" is the measured concentration of an active agent in the plasma at about n hours after administration. "$C_{24}$" is the measured concentration of an active agent in the plasma at about 24 hours after administration. The term "$T_{max}$" refers to the time at which the measured concentration of an active agent in the plasma is the highest after administration of the active agent. "AUC" is the area under the curve of a graph of the measured concentration of an active agent (typically plasma concentration) vs. time, measured from one time point to another time point. For example $AUC_{0-t}$ is the area under the curve of plasma concentration versus time from time 0 to time t. The $AUC_{0-\infty}$ or $AUC_{0-INF}$ is the calculated area under the curve of plasma concentration versus time from time 0 to time infinity.

"Phenotype" means an observable trait of an organism resulting from the interplay of environment and genetics. Examples include apparent rate of metabolism of substrates by a cytochrome p450 isozyme of an organism, such as the "poor metabolizer" (PM) or "ultrarapid metabolizer" (UM) phenotypes identified in humans for metabolism of substrates metabolized by CYP2D6.

"Polymorphism" means the differences in DNA sequences that occur naturally in a population. Single nucleotide substitutions, insertions, and deletions of nucleotides and repetitive sequences (microsatellites) are all examples of a polymorphism.

A "product" or "pharmaceutical product" means a dosage form of an active agent plus published material, and optionally packaging.

"Providing" means giving, administering, selling, distributing, transferring (for profit or not), manufacturing, compounding, or dispensing.

"Published material" means a medium providing information, including printed, audio, visual, or electronic medium, for example a flyer, an advertisement, a product insert, printed labeling, an internet web site, an internet web page, an internet pop-up window, a radio or television broadcast, a compact disk, a DVD, an audio recording, or other recording or electronic medium.

"Safety" means the incidence or severity of adverse events associated with administration of an active agent, including adverse effects associated with patient-related factors (e.g., age, gender, ethnicity, race, target illness, abnormalities of renal or hepatic function, co-morbid illnesses, genetic characteristics such as metabolic status, or environment) and active agent-related factors (e.g., dose, plasma level, duration of exposure, or concomitant medication).

"Salts" as used herein describes "pharmaceutically acceptable salts" of metaxalone and other active agents discussed herein and also includes solvates and hydrates of such active agents. The active agent may be modified by making non-toxic acid or base addition salts thereof. Examples of pharmaceutically acceptable salts include mineral or organic acid addition salts of basic residues such as amines; alkali or organic addition salts of acidic residues; and the like, and combinations comprising one or more of the foregoing salts. The pharmaceutically acceptable salts include non-toxic salts and the quaternary ammonium salts of the metaxalone. For example, non-toxic acid salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; other acceptable inorganic salts include metal salts such as sodium salt, potassium salt, cesium salt, and the like; and alkaline earth metal salts, such as calcium salt, magnesium salt, and the like, and combinations comprising one or more of the foregoing salts. Pharmaceutically acceptable organic salts includes salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, mesylic, esylic, besylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, HOOC—$(CH_2)_n$—COOH where n is 0–4, and the like; organic amine salts such as triethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, and the like; and amino acid salts such as arginate, asparginate, glutamate, and the like; and combinations comprising one or more of the foregoing salts.

Solid dosage forms of metaxalone comprise up to about 3600 mg metaxalone, specifically about 50 to about 3200 mg metaxalone, more specifically about 100 to about 800 mg metaxalone. In one embodiment, the solid dosage form is an oral dosage form, for example, a tablet.

A "substance" taken or administered with metaxalone means a substance that affects the safety, bioavailability, plasma concentration, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. A "substance" can be an active agent, an herbal supplement, a nutritional supplement, a vitamin, a xenobiotic, or an environmental contaminant.

A substance is a "substrate" of enzyme activity when it can be chemically transformed by action of the enzyme on the substance. "Enzyme activity" refers broadly to the specific activity of the enzyme (i.e., the rate at which the enzyme transforms a substrate per mg or mole of enzyme) as well as the metabolic effect of such transformations. Thus, a substance is an "inhibitor" of enzyme activity when the specific activity or the metabolic effect of the specific activity of the enzyme can be decreased by the presence of the substance, without reference to the precise mechanism of such decrease. For example a substance can be an inhibitor of enzyme activity by competitive, non-competitive, allosteric or other type of enzyme inhibition, by decreasing expression of the enzyme, or other direct or indirect mechanisms. Similarly, a substance is an "inducer" of enzyme activity when the specific activity or the metabolic effect of the specific activity of the enzyme can be increased by the presence of the substance, without reference to the precise mechanism of such increase. For example a substance can be an inducer of enzyme activity by increasing reaction rate, by increasing expression of the enzyme, by allosteric activation or other direct or indirect mechanisms. It is possible for a substance to be a substrate, inhibitor, or inducer of an

enzyme activity. For example, the substance can be an inhibitor of enzyme activity by one mechanism and an inducer of enzyme activity by another mechanism. The function (substrate, inhibitor, or inducer) of the substance with respect to activity of an enzyme can depend on environmental conditions.

A "user" means a patient, a medical care worker, or a pharmaceutical supplier.

The cytochrome p450 enzymes are a highly diverse superfamily of enzymes. Each cytochrome p450 enzyme is termed an "isoform" or "isozyme" since each derives from a different gene. Cytochrome p450 enzymes are categorized into families and subfamilies by amino acid sequence similarities. These enzymes are designated by the letters "CYP" followed by an Arabic numeral representing the family, a letter representing the sub-family and another Arabic numeral representing a specific gene (e.g., CYP2D6). Particular isozymes discussed herein are named as per the recommendations of the P450 Gene Superfamily Nomenclature Committee (see e.g., "P450 superfamily: Update on new sequences, gene mapping, accession numbers, and nomenclature" Pharmacogenetics 6, 1-42 1996, part A pp. 1-21.). Herein, the designation for a cytochrome p450 isozyme may encompass the homolog from any species identified as having such an isozyme. For example, CYP1A2 genes are known in at least rat, human, rabbit, hamster, dog, guinea pig, mouse and chicken and the designation "CYP1A2" includes the CYP1A2 protein from each species known to have a CYP1A2 gene. In some embodiments, the designation for a cytochrome p450 isozyme is the human isozyme.

In one embodiment, CYP1A2 is human CYP1A2 (Entrez Gene ID: 1544; reference protein sequence Genbank NP_000752), and includes any allelic variants. Specifically, CYP1A2 includes any allelic variants included in the list of human CYP1A2 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *16 alleles. Additional reference amino acid sequences for human CYP1A2 include Genbank AAK25728, AAY26399, AAA35738, AAA52163, AAA52163, AAF13599, AAH67424, AAH67425, AAH67426, AAH67427, AAH67428, AAH67429, AAA52154, AAA52146, CAA77335, P05177, Q6NWU3, Q6NWU5, Q9BXX7, and Q9UK49.

In one embodiment, CYP2A6 is human CYP2A6 (Entrez Gene ID: 1548; reference protein sequence Genbank NP_000753), and includes any CYP2A6 allelic variants. Specifically, CYP2A6 includes any allelic variants included in the list of human CYP2A6 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *22 alleles. Additional reference amino acid sequences for human CYP2A6 include Genbank AAG45229, AAB40518, AAF13600, AAH96253, AAH96254, AAH96255, AAH96256, AAA52067, CAA32097, CAA32117, P11509, Q13120, and Q4VAU0.

In one embodiment, CYP2B6 is human CYP2B6 (Entrez Gene ID: 1555; reference protein sequence Genbank NP_000758), and includes any CYP2B6 allelic variants. Specifically, CYP2B6 includes any allelic variants included in the list of human CYP2B6 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *25 alleles. Additional reference amino acid sequences for human CYP2B6 include Genbank AAF32444, AAD25924, ABB84469, AAF13602, AAH67430, AAH67431, AAA52144, P20813, Q6NWU1, Q6NWU2, and Q9UNX8.

In one embodiment, CYP2C8 is human CYP2C8 (Entrez Gene ID: 1558; reference protein sequence Genbank NP_110518), and includes any CYP2C8 allelic variants. Specifically, CYP2B8 includes any allelic variants included in the list of human CYP2C8 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *10 alleles. Additional reference amino acid sequences for human CYP2C8 include Genbank CAH71307, AAR89907, CAA38578, AAH20596, AAA35739, AAA35740, AAA52160, AAA52161, CAA35915, CAA68550, P10632, Q5VX93, Q8WWB1, and Q9UCZ9.

In one embodiment, CYP2C9 is human CYP2C9 (Entrez Gene ID: 1559; reference protein sequence Genbank NP_000762), and includes any CYP2C9 allelic variants. Specifically, CYP2CP includes any allelic variants included in the list of human CYP2C9 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *24 alleles. Additional reference amino acid sequences for human CYP2C9 include Genbank CAH71303, AAP88931, AAT94065, AAW83816, AAD13466, AAD13467, AAH20754, AAH70317, BAA00123, AAA52159, AAB23864, P11712, Q5EDC5, Q5VX92, Q6IRV8, Q8WW80, Q9UEH3, and Q9UQ59.

In one embodiment, CYP2C19 is human CYP2C19 (Entrez Gene ID: 1557; reference protein sequence Genbank NP_000760), and includes any CYP2C19 allelic variants. Specifically, CYP2C19 includes any allelic variants included in the list of human CYP2C19 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *21 alleles. Additional reference amino acid sequences for human CYP2C19 include Genbank BAD02827, CAH73444, CAH74068, AAV41877, AAL31347, AAL31348, AAA36660, AAB59426, CAA46778, P33261, Q16743, Q767A3, Q8WZB1, and Q8WZB2.

In one embodiment, CYP2D6 is human CYP2D6 (Entrez Gene ID: 1565; reference protein sequence Genbank NP_000097), and includes any CYP2D6 allelic variants. Specifically, it CYP2D6 includes any allelic variants included in the list of human CYP2D6 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *58 alleles. Additional reference amino acid sequences for human CYP2D6 include Genbank AAS55001, ABB01370, ABB01371, ABB01372, ABB01373, AAA35737, AAA53500, BAD92729, AAU87043, AAH66877, AAH67432, AAH75023, AAH75024, AAI06758, AAI06759, CAG30316, AAA52153, AAA36403, CAA30807, and P10635.

In one embodiment, CYP2E1 is human CYP2E1 (Entrez Gene ID: 1571; reference protein sequence Genbank NP_000764), and includes any CYP2E1 allelic variants. Specifically, CYP2E1 includes any allelic variants included in the list of human CYP2E1 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *7 alleles. Additional reference amino acid sequences for human CYP2E1 include Genbank CAH70047, BAA00902, BAA08796, AAA52155, AAD13753, AAF13601, CAI47002, AAH67433,

AAH67435, AAZ77710, AAA35743, AAD14267, $P_{05181}$, Q16868, Q5VZD5, Q6LER5, Q6NWT7, and Q6NWT9.

In one embodiment, CYP3A4 is human CYP3A4 (Entrez Gene ID: 1576; reference protein sequence Genbank NP_059488), and includes any CYP3A4 allelic variants. Specifically, CYP3A4 includes any allelic variants included in the list of human CYP3A4 allelic variants maintained by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee; more specifically it includes any of the *1 through *20 alleles. Additional reference amino acid sequences for human CYP3A4 include Genbank AAF21034, AAG32290, AAG53948, EAL23866, AAF13598, CAD91343, CAD91645, CAD91345, AAH69418, AAI01632, BAA00001, AAA35747, AAA35742, AAA35744, AAA35745, CAA30944, P05184, P08684, Q6GRK0, Q7Z448, Q86SK2, Q86SK3, and Q9BZM0.

The ability of metaxalone to act as a substrate, inhibitor, or inducer of various cytochrome p450 isozymes was determined in studies described below. A summary of the findings of the studies is provided in Table 1.

TABLE 1

Summary of metaxalone effects on cytochrome p450 isozymes.

| CYP isozyme | Substrate | Inhibitor | Inducer/Inhibitor |
|---|---|---|---|
| 1A2 | + | + | + |
| 2A6 | 0 | 0 | 0 |
| 2B6 | ND | + | 0 |
| 2C8 | ND | 0 | ND |
| 2C9 | 0 | 0 | – |
| 2C19 | + | + | 0 |
| 2D6 | 0 | + | – |
| 2E1 | 0 | + | 0 |
| 3A4 | 0 | + | + |

For each possible function of metaxalone (i.e., substrate, inhibitor, or inducer), there is a column in the table. A "+" in a particular column and row indicates that the study found that metaxalone functioned in that capacity with respect to the cytochrome p450 isozyme represented in that row, while a "0" indicates that the results did not support that metaxalone functioned in that capacity with respect to the cytochrome p450 isozyme represented in that row. In the column labeled Inducer/Inhibitor, a "+" denotes that the metaxalone functioned as an inducer of the CYP isozyme, while a "–" denotes that metaxalone functioned as an inhibitor of the CYP isozyme. For example, metaxalone was found to be a substrate, inhibitor, and inducer of CYP1A2 activity, and was found to be an inhibitor of CYP2C9 activity. The symbol "ND" indicates that no experiment was performed.

As summarized in Table 1, metaxalone was found to be a substrate for CYP2C19 and CYP1A2, and therefore can also act as a competitor of other substrates for these two isozymes. Additionally, metaxalone was determined to be an inhibitor of the cytochrome p450 isozymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 and an inducer of CYP1A2 and CYP3A4.

Enzymes involved in Phase I and Phase II active agent metabolism, such as the cytochrome p450 isozymes, respond to the constantly changing types and amounts of substrate active agents they encounter. For example, changes in active agent metabolism due to competition for the same cytochrome p450 isoform can change the clinical effectiveness or safety of an active agent by altering the plasma concentration of the active agent or its metabolite(s). Similarly, inhibition or induction of the cytochrome p450 isoform that metabolizes a particular active agent can change the clinical effectiveness or safety of that active agent. Therefore, for any cytochrome p450 for which metaxalone acts as a substrate, inhibitor, or inducer, the administration of metaxalone with a substance that is a substrate, inhibitor, or inducer of that cytochrome p450 can affect the metabolism of the metaxalone or the substance. For the case in which the substance is a narrow therapeutic index active agent, such as warfarin or phenytoin, too little of the active agent in the blood stream can lead to insufficient therapeutic activity, while a too large dose of the active agent can lead to excessive therapeutic activity or toxicity, both of which can be detrimental.

The invention provides methods of using metaxalone. These methods include using metaxalone in the treatment of various diseases or conditions, including, for example, musculoskeletal conditions, specifically acute and painful musculoskeletal conditions, muscle sprains, muscle spasms, spasticity, low back pain and stiffness, acute lumbosacral pain, cervical stiffness or torticohis; as well as head pain, including migraines, cluster headaches, tension headaches, or tension related migraines.

In one embodiment, the method comprises informing a user that metaxalone affects activity of a cytochrome p450 isozyme. The cytochrome p450 isozyme may be any cytochrome p450 isozyme. For example the cytochrome p450 isozyme may be CYP1A2 CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. In some embodiments the cytochrome p450 isozyme is CYP1A2, CYP3A4, or CYP2C19. In certain embodiments the cytochrome p450 isozyme is a human enzyme. In some embodiments, the method further comprises providing the user with metaxalone.

Informing the user that metaxalone affects the activity of a cytochrome p450 isozyme includes providing a user with information about any effect of metaxalone on the activity of any cytochrome p450 isozyme. Informing the user that metaxalone affects the activity of a cytochrome p450 isozyme includes informing a user of any of the following: that metaxalone is metabolized by a cytochrome p450 isozyme; that metaxalone is an inducer of activity of a cytochrome p450 isozyme; that a cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19; that metaxalone is a competitive inhibitor of CYP1A2 or CYP2C19; that metaxalone is a substrate of CYP1A2 or CYP2C19; that there is a potential active agent interaction between metaxalone and an active agent that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19; that metaxalone is an inhibitor of a cytochrome p450 isozyme; that caution is recommended when metaxalone and a substrate of CYP2B6, CYP2C9, CYP2C19, or CYP2D6 are administered to a patient known to have a poor metabolizer phenotype for or that has reduced activity of CYP2B6, CYP2C9, CYP2C19, or CYP2D6; that caution is recommended when administering metaxalone with the substance when the substance is an active agent having a narrow therapeutic index; that the allelic variants of CYP2B6, CYP2C9, CYP2C19, or CYP2D6 present in the patient can further affect the potential active agent interaction between metaxalone and an active agent; that there is a potential active agent interaction of metaxalone with an active agent that is a substrate of the cytochrome p450 isozyme; that there is a potential active agent interaction of metaxalone with warfarin; that metaxalone affects the activity of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4; that there is a potential active agent interaction of metaxalone with a substance that is a substrate of CYP1A2, CYP2B6,

CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4; that metaxalone is an inhibitor of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4; and that metaxalone is an inducer of CYP1A2 or CYP3A4 activity; that there is a potential active agent interaction of metaxalone with a substance that is a substrate of CYP1A2 or CYP3A4.

The method can further comprise informing the user that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. In some embodiments, the method further comprises providing the user with the substance.

The effect of coadministration of metaxalone and the substance can be determined by comparison of the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance with and without coadministration of metaxalone or by comparison of the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone with and without coadministration of the substance.

Informing the user that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance includes providing a user with information about any effect of metaxalone on plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. This includes informing a user of any of the following: that taking metaxalone with an active agent can affect the bioavailability, safety, or efficacy of the active agent or metaxalone; that administration of metaxalone and a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance; that administration of metaxalone with a substance that is a CYP1A2 or CYP2C19 substrate can increase the plasma concentration of the substance; that taking metaxalone with an active agent that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the active agent; that administration of metaxalone with an active agent that is a cytochrome p450 isozyme substrate having a narrow therapeutic index can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent; that administration of metaxalone with an active agent that is a CYP1A2 or CYP2C19 substrate having a narrow therapeutic index can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent; that metaxalone can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of an active agent that is a substrate of the cytochrome p450 isozyme; that administration of metaxalone with an active agent that is a substrate of the cytochrome p450 isozyme and that has a narrow therapeutic index can increase plasma concentration of the active agent; that a substance that induces CYP1A2 or CYP2C19 activity can decrease metaxalone plasma concentration; that a substance that inhibits CYP1A2 or CYP2C19 activity can increase metaxalone plasma concentration; that a substance that is a substrate of CYP1A2 or CYP2C19 can increase plasma concentration of metaxalone or the substance; that administration of metaxalone with warfarin can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or warfarin; that administration of metaxalone with an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of CYP2B6, CYP2C9, CYP2D6, CYP2E1, or CYP3A4 metaxalone can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or metaxalone; that the plasma concentration of a substance that is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 can decrease when the substance is administered with metaxalone; that administration of metaxalone with a substance that is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance; that administration of metaxalone with an active agent that is a CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 substrate having a narrow therapeutic index can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent; that the plasma concentration of a substance that is a substrate of CYP1A2 or CYP3A4 can decrease when the substance is administered with metaxalone; that administration of metaxalone and a substance that is a substrate of CYP1A2 or CYP3A4 activity can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance.

In another embodiment, the method comprises informing a user that metaxalone is metabolized by a cytochrome p450 isozyme. The cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19. In some embodiments, the method further comprises informing the user that administration of metaxalone and a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. Methods provided herein include informing a user that the substance or metaxalone is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19. The substance can inhibit CYP1A2 or CYP2C19 activity and the effect can be an increase in metaxalone plasma concentration, or the substance can induce CYP1A2 or CYP2C19 activity and the effect can be a decrease in metaxalone plasma concentration. In yet another embodiment, the user is informed that the substance is a substrate of CYP1A2 or CYP2C19 and plasma concentration of the substance or metaxalone can increase. In yet another embodiment, the method comprises informing the user that taking metaxalone and a substance that is a substrate of CYP1A2 or CYP2C19 can increase plasma concentration of metaxalone or the substance.

The method also comprises informing a user that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme. Cytochrome p450 isozymes inhibited by metaxalone include CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Cytochrome p450 isozymes that are induced by metaxalone include CYP1A2 and CYP3A4. In some embodiments the method further comprises informing a user that administration of metaxalone and a substance that is a substrate of the cytochrome p450 isozyme can affect plasma concentration, bioavailabil-

ity, safety, efficacy, or a combination comprising at least one of the foregoing of the substance. In some embodiments, the method can further comprise informing that metaxalone is an inhibitor of the cytochrome p450 isozyme or that the effect on the substance can be an increase in plasma concentration. In other embodiments, the method can further comprise informing that metaxalone is an inducer of CYP1A2 or CYP3A4 or that the effect on the substance can be a decrease in plasma concentration.

In some embodiments, the method can further comprise providing the user with metaxalone. Other embodiments include administering metaxalone or another substance. Administration may be to a patient by the patient, a medical worker, or other user. Metaxalone can be administered in a therapeutically effective amount. In some embodiments, the method can further comprise providing the user with metaxalone or informing the user that caution is recommended when administering metaxalone with the substance when the substance is an active agent having a narrow therapeutic index.

The information provided to a user can comprise any combination of information disclosed herein concerning the effects of metaxalone on the activity of a cytochrome p450 isozyme or on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or a substance. The information may also comprise any combination of information disclosed herein concerning the effects of a substance on the activity of a cytochrome p450 isozyme or on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or a substance when the substance is used with metaxalone.

Medical information provided in any of the methods described herein concerning the effects of administering metaxalone with an additional substance may alternatively be provided in layman's terms, so as to be better understood by patients or non-medical professionals. Those of skill in the medical art are familiar with the various layman's terms that can be used to describe the effects of active agent interactions.

In yet another embodiment, the method of using metaxalone comprises obtaining metaxalone from a container providing information that metaxalone affects activity of a cytochrome p450. Information can also be provided that administering metaxalone with a substance can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance or metaxalone. The method also comprises providing metaxalone in the container providing such information. The method may also comprise providing a substance, such as an active agent, in a container providing information that metaxalone affects activity of a cytochrome p450 or that administering metaxalone with the substance may affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance or metaxalone. The provided information may be any information disclosed herein concerning the effects of metaxalone or a substance on the activity of a cytochrome p450 isozyme or any information disclosed herein concerning the effects of metaxalone when administered with a substance on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance or metaxalone. The method can further comprise ingesting the metaxalone or the substance.

The method of use can further comprise monitoring a patient's plasma concentration of an active agent as $AUC_{0-INF}$, $AUC_{0-t}$, $C_{MAX}$, or a combination of any of the foregoing pharmacokinetic parameters.

The method may also comprise informing the user or providing information that, when an active agent and metaxalone are administered to a patient, that it is recommended that a medical care worker determine the patient's plasma concentration of the active agent; and alter dosing of the active agent for the patient based on the determined active agent plasma concentration. Additionally, the method can comprise determining the metabolizer phenotype of the patient or the allelic variant of the patient for a cytochrome p450 isozyme; specifically the cytochrome p450 isozyme is CYP2B6, CYP2C9, CYP2C19, or CYP2D6.

Various laboratory methods are known, including ones that are commercially available, for detecting the presence of allelic variants of cytochrome p450 isozymes in an individual or determining the metabolizer phenotype of an individual for a particular cytochrome p450 isozyme. Any suitable method known in the art may be used. Methods include analyzing a blood sample from the individual to determine the allelic variant of a particular cytochrome p450 isozyme gene present in the individual (for example by genotyping or haplotyping DNA or RNA from the gene using mass spectrometry, gel electrophoresis, or TAQMAN assays; or analyzing the protein sequence expressed by the gene). The metabolizer phenotype of the individual can be inferred based on the known properties of the allelic variants determined to be present in the individual. Alternatively, the blood sample can be used to measure enzyme activity of the cytochrome p450 isozyme using a suitable assay amd isozyme-selective substrate. Among suitable isozyme-selective substrates are those used in the studies herein, or those suggested in FDA guidelines directed to collecting cytochrome p450 isozyme data for regulatory submissions relating to an active agent.

Food may alter the release of an active agent from a dosage form, the solubilization of the active agent, and the transport of the active agent across the intestinal wall. According to U.S. Pat. No. ,6407,128, pharmacokinetic studies of metaxalone indicate that food increases the rate and extent of absorption of a 400 mg oral dosage form in humans. In that study, food increased peak plasma concentrations ($C_{max}$), and extent of absorption ($AUC_{0-t}$, $AUC_{0-inf}$) relative to a fasted treatment with observed increases of 177.5%, 123.5%, and 115.4%. Based on that study, administration of metaxalone with food increases the bioavailability of metaxalone and therefore a particular oral dose given with food may physiologically correspond to a higher plasma concentration of metaxalone than the same oral dose given in a fasted state. Consequently, any effect on plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of administration of metaxalone with an additional substance which is a substrate, inhibitor, or inducer of a cytochrome p450 isozyme for which metaxalone is a substrate, inhibitor, or inducer can be further affected by whether or not the metaxalone was administered with food.

Methods of using metaxalone comprise informing a user that metaxalone affects the activity of a cytochrome p450; that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of

metaxalone or the substance can vary with administration of metaxalone with or without food.

In another embodiment, the method of using metaxalone comprises obtaining metaxalone from a container providing information that metaxalone affects activity of a cytochrome p450; that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, or efficacy of metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, or efficacy of metaxalone or the substance can vary with administration of metaxalone with or without food. The method also includes providing metaxalone in the container providing information.

In one embodiment, the metaxalone is always administered with food. In another embodiment, the metaxalone is always administered without food. In yet another embodiment, the metaxalone is sometimes administered with food and sometimes administered without food.

Also disclosed herein are methods of manufacturing a metaxalone pharmaceutical product.

In one embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone affects activity of a cytochrome p450 isozyme. The information may also advise that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. The information may also include any information disclosed herein about the effect of metaxalone or a substance on the activity of a cytochrome p450 isozyme and any information disclosed herein about the effect of metaxalone or a substance on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance.

In an embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone is metabolized by a cytochrome p450 isozyme. The cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19. The information may also advise that administration of metaxalone and a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance.

In an embodiment, the method comprises packaging a metaxalone dosage form with information that administration of metaxalone with an active agent that is a CYP1A2 or CYP2C19 substrate having a narrow therapeutic index can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent.

In another embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme. The information may further advise that administration of metaxalone with an active agent that is a substrate of the cytochrome p450 isozyme can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent. The cytochrome p450 isozyme is CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. In some embodiments, the active agent is a substrate of the cytochrome p450 isozyme inhibited by metaxalone and the plasma concentration of the active agent can increase; in other embodiments, the active agent is a substrate of the cytochrome p450 isozyme induced by metaxalone and the plasma concentration of the active agent can decrease.

In yet another embodiment, the method comprises packaging a metaxalone dosage form with information that metaxalone affects activity of a cytochrome p450 isozyme and that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance can vary with administration of metaxalone with or without food. In one embodiment, the metaxalone is always administered with food. In another embodiment, the metaxalone is always administered without food. In yet another embodiment, the metaxalone is sometimes administered with food and sometimes administered without food.

Another aspect of the invention is a method of using an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme.

In one embodiment, the method comprises informing a user that metaxalone affects activity of a cytochrome p450 isozyme and that administration of the active agent and metaxalone can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or the metaxalone. The cytochrome p450 isozyme is CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. In some embodiments, the method further comprises providing the user with the active agent or metaxalone.

In another embodiment, the method comprises obtaining an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme from a container providing information that metaxalone affects activity of a cytochrome p450 isozyme and that the administration of the active agent with metaxalone can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or the metaxalone. The method may also comprise providing the active agent in the container providing information.

Also disclosed herein is a method of manufacturing a pharmaceutical product of an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme.

In one embodiment, the method comprises packaging a dosage form of an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme with information that metaxalone affects activity of a cytochrome p450 isozyme.

In each of the methods for using an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme or the methods of manufacturing a pharmaceutical product of such an active agent, the information provided to the user or with the dosage form may include any information disclosed herein about the effect of metaxalone or the active agent on the activity of a cytochrome p450 isozyme and any information disclosed herein about the effect of metaxalone or the active agent on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the active agent.

The invention provides articles of manufacture.

In some embodiments, the article of manufacture comprises a container containing a dosage form of metaxalone.

In one embodiment, the container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme. The published material can further inform that administration of metaxalone with a substance that is a substrate, inhibitor, or inducer of the cytochrome p450 isozyme can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. The published material may be in the form of printed labeling, or in some other form. The cytochrome p450 can be CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. The published material comprising the article of manufacture may also include any information disclosed herein about the effect of metaxalone or a substance on the activity of a cytochrome p450 isozyme and any information disclosed herein about the effect of metaxalone or a substance on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance.

In another embodiment, the container is associated with published material informing that metaxalone is metabolized by a cytochrome p450 isozyme. The cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19. In some embodiments, the published material further informs that administration of metaxalone with a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. In other embodiments, the published material further informs that a substance that induces CYP1A2 or CYP2C19 activity can decrease metaxalone plasma concentration, that a substance that inhibits CYP1A2 or CYP2C19 activity can increase metaxalone plasma concentration, or that a substance that is a substrate of CYP1A2 or CYP2C19 can increase plasma concentration of metaxalone or the substance.

In yet another embodiment, the container is associated with published material informing that metaxalone is an inhibitor or an inducer of a cytochrome p450 isozyme. The published material may further inform that administration of metaxalone can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of substances that are substrates of the cytochrome p450 isozyme. The cytochrome p450 isozyme is CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.

In another embodiment, the container is associated with published material that includes information that caution is recommended when administering metaxalone with the substrate, wherein the substrate has a narrow therapeutic index.

In yet another embodiment, the container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme; that administration to a patient of metaxalone with a substance can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance; and that any effect on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance can vary with administration of metaxalone with or without food.

In yet another embodiment, the article comprises a container comprising a dosage form of metaxalone, and published material. In one embodiment, the published material provides information that there is a potential active agent interaction with warfarin; or that administration with warfarin can affect the bioavailability, safety, or efficacy of metaxalone or warfarin. In another embodiment, the published material informs that metaxalone affects activity of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. The published material may further inform that there is a potential active agent interaction with a substance that is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 or that administration of metaxalone with a substance that is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance. In another embodiment, the published material informs that metaxalone is a substrate of CYP1A2 or CYP2C19. The published material may also inform that there is a potential active agent interaction with a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that administration of metaxalone with a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. In yet another embodiment, the published material informs that metaxalone is an inhibitor of activity of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. In yet another embodiment, the published material informs that metaxalone is an inducer of activity of CYP1A2 or CYP3A4. In each of these latter embodiments, the published material may further inform that there is a potential active agent interaction with a substance that is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 or that administration of metaxalone with a substance that is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the substance. In some embodiments, the published material can be printed labeling.

Also disclosed herein is an article of manufacture comprising packaging material and a dosage form contained within the packaging material, wherein the dosage form comprises, as at least one active ingredient, metaxalone, and wherein the packaging material comprises a label approved by a regulatory agency for the product. The label may inform that metaxalone affects activity of a cytochrome p450 isozyme; that a cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19; that metaxalone is an inhibitor of activity of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4; or that metaxalone is an inducer of activity of CYP1A2 or CYP3A4. Examples of regulatory agencies are the US FDA or the European Agency for the Evaluation of Medicinal Products (EMEA).

The invention further includes an article of manufacture comprising a container holding a dosage form of metaxalone associated with published material informing that there is a potential active agent interaction with warfarin, or that administration with warfarin can affect the bioavailability, safety, or efficacy of the metaxalone or the warfarin. The published material may further comprise instructions regarding measuring the Prothrombin Time/International Normalized Ratio daily, every other day, weekly, every other week, monthly, or according to another schedule or time criteria, or instructions to monitor the blood levels of warfarin as $AUC_{0-t}$, $AUC_{0-INF}$, $C_{MAX}$, or a combination comprising one or more of the foregoing pharmacokinetic parameters.

The invention includes articles of manufacture in which the substance administered with metaxalone is phenytoin. In one embodiment, the article of manufacture comprises a container holding a dosage form of metaxalone associated with published material informing that there is a potential active agent interaction with phenytoin, or that administration of metaxalone with phenytoin can affect the bioavailability, safety, efficacy or a combination comprising at least one of the foregoing of the metaxalone or the phenytoin. The published material may further comprise instructions to monitor the blood levels of phenytoin as $AUC_{0-t}$, $AUC_{0-INF}$, $C_{MAX}$, or a combination comprising one or more of the foregoing pharmacokinetic parameters.

Also disclosed herein is an article of manufacture comprising a container containing a dosage form of an active agent that is a known substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme. The container is associated with published material informing that metaxalone affects activity of a cytochrome p450 isozyme and administration to a patient of the active agent and metaxalone can affect plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of the active agent or metaxalone. In one embodiment of any of these methods or articles involving an active agent that is a known substrate, inhibitor or inducer of CYP1A2 or CYP2C19 or that is a substrate of a cytochrome p450 isozyme, the active agent is an inducer of CYP1A2 or CYP2C19 and plasma concentration of metaxalone can decrease. In another embodiment the active agent is an inhibitor of CYP1A2 or CYP2C19 and plasma concentration of metaxalone can increase. In yet another embodiment, the active agent is a substrate of CYP1A2 or CYP2C19 and plasma concentration of the active agent and/or metaxalone can increase. In yet another embodiment, the active agent is a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 and plasma concentration of the active agent can increase. In yet another embodiment, the active agent is a substrate of CYP1A2 or CYP3A4 and plasma concentration of the active agent can decrease. In any of these embodiments, the active agent can have a narrow therapeutic index. The published material comprising the article of manufacture may also include any information disclosed herein about the effect of metaxalone or the active agent on the activity of a cytochrome p450 isozyme and any information disclosed herein about the effect of metaxalone or the active agent on the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the active agent.

In embodiments of the articles of manufacture, the dosage form will typically be contained in a suitable container capable of holding and dispensing the dosage form and which will not significantly interact with the active agent(s) in the dosage form. Further, the container will be in physical relation with the published material. The published material may be associated with the container by any means that maintains physical proximity of the two. By way of example, the container and the published material can both be contained in a packaging material such as a box or plastic shrink wrap. Alternatively, the published material can be bonded to the container, such as with glue that does not obscure the published material, or with other bonding or holding means. Yet another alternative is that the published material is placed within the container with the dosage form.

In other embodiments of the article, someone hands the published material to the patient, for example a pharmacist can hand a product insert to a patient in conjunction with dispensing the dosage form. The published material may be a product insert, flyer, brochure, or a packaging material for the dosage form such as a bag, or the like.

In any of the embodiments disclosed herein the published material or information associated with or provided by a container can be contained in any fixed and tangible medium. For example, the information can be part of a leaflet, brochure, or other printed material provided with a container or separate from a container. The information can also take the form of a flyer, advertisement, or the label for marketing the active agent approved by a regulatory agency. The information can also be recorded on a compact disk, DVD or any other recording or electronic medium.

The substance used with metaxalone in the methods and articles of manufactures described herein may have certain effects, direct or indirect, on the activity of a cytochrome p450 enzyme. The substance or metaxalone can be a substrate, inhibitor, or inducer of a Phase I or Phase II metabolic enzyme; specifically, the substance or metaxalone is a substrate, inhibitor, or inducer of a cytochrome p450 isozyme. More specifically, the substance can be a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4, or an inhibitor or inducer of CYP1A2 or CYP2C19. Metaxalone can be a substrate, inhibitor, or inducer of CYP1A2; a substrate or inhibitor of CYP2C19; an inhibitor of CYP2B6, CYP2C9, CYP2D6, or CYP2E1; or an inhibitor or inducer of CYP3A4. For example in certain embodiments the substance is: a substrate, inhibitor, or inducer of a cytochrome p450 isozyme; an active agent; a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 activity; an active agent with a narrow therapeutic index; an inducer of CYP1A2 or CYP2C19 activity and plasma concentration of metaxalone can decrease; an inhibitor of CYP1A2 or CYP2C19 and plasma concentration of metaxalone can increase; a substrate of CYP1A2 or CYP2C19 and plasma concentration of the substance or metaxalone can increase; a substrate of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 and plasma concentration of the substance can increase; or the substance is a substrate of CYP1A2 or CYP3A4 and plasma concentration of the substance can decrease when the substance is administered with metaxalone.

In any of the above methods or articles, the substance can be an active agent.

Examples of active agents that are substrates of CYP1A2 include amitriptyline, caffeine, clomipramine, clozapine, cyclobenzaprine, estradiol, fluvoxamine, haloperidol, imipramine, mexiletine, naproxen, olanzapine, ondansetron, phenacetin, acetaminophen, propranolol, riluzole, ropivacaine, tacrine, theophylline, tizanidine, verapamil, (R)-warfarin, zileuton, and zolmitriptan. Examples of active agents that are inhibitors of CYP1A2 include amiodarone, cimetidine, fluoroquinolones, fluvoxamine, furafylline, interferon, methoxsalen, and mibefradil. Examples of inducers of CYP1A2 include insulin, methyl cholanthrene, modafinil, nafcillin, beta-naphthoflavone, omeprazole, and tobacco.

Examples of active agents that are substrates of CYP2C19 include the proton pump inhibitors: lansoprazole, omeprazole, pantoprazole, and E-3810; the anti-epileptics: diazepam, phenytoin, fosphenytoin, S-mephenytoin, and phenobarbitone (Phenobarbital); as well as amitriptyline, carisoprodol, citalopram, clomipramine, cyclophosphamide, hexobarbital, imipramine, indomethacin, R-mephobarbital, moclobemide, nelfinavir, nilutamide, primidone, progesterone, proguanil, propranolol, teniposide, and R-warfarin.

Examples of active agents that are inhibitors of CYP2C19 include chloramphenicol, cimetidine, felbamate, fluoxetine, fluvoxamine, indomethacin, ketoconazole, lansoprazole, modafinil, omeprazole, oxcarbazepine, probenicid, ticlopidine, and topiramate. Examples of inducers of CYP2C19 include carbamazepine, norethindrone, prednisone, and rifampin (rifampicin).

Examples of active agents that are substrates of CYP2B6 include bupropion, cyclophosphamide, efavirenz, ifosfamide, and methadone.

Examples of active agents that are substrates of CYP2C9 include diclofenac, ibuprofen, meloxicam, S-naproxen, piroxicam, suprofen, tolbutamide, glipizide, losartan, irbesartan, glyburide (glibenclamide), glipizide, glimepiride, amitriptyline, celecoxib, fluoxetine, fluvastatin, nateglinide, phenytoin, rosiglitazone, tamoxifen, torsemide, and S-warfarin.

Examples of active agents that are substrates of CYP2D6 include carvedilol, S-metoprolol, propafenone, timolol; amitriptyline, clomipramine, desipramine, imipramine, paroxetine; haloperidol, perphenazine, risperidone, thioridazine; alprenolol, amphetamine, aripiprazole, atomoxetine, bufuralol, chlorpheniramine, chlorpromazine, codeine, debrisoquine, dexfenfluramine, dextromethorphan, duloxetine, encainide, flecainide, fluoxetine, fluvoxamine, lidocaine, metoclopramide, methoxyamphetamine, mexiletine, minaprine, nebivolol, nortriptyline, ondansetron, perhexiline, phenacetin, phenformin, propranolol, sparteine, tamoxifen, tramadol, and venlafaxine.

Examples of substrates of CYP2E1 include enflurane, halothane, isoflurane, methoxyflurane, sevoflurane; acetaminophen, aniline, benzene, chlorzoxazone, ethanol, N,N-dimethyl formamide, and theophylline.

Examples of substrates of CYP3A4 include clarithromycin, erythromycin, telithromycin: quinidine; alprazolam, diazepam, midazolam, triazolam; cyclosporine, tacrolimus (FK506); indinavir, nelfinavir, ritonavir, saquinavir; cisapride; astemizole, chlorpheniramine, terfenadine; amlodipine, diltiazem, felodipine, lercanidipine, nifedipine, nisoldipine, nitrendipine, verapamil; atorvastatin, cerivastatin, lovastatin, simvastatin; estradiol, hydrocortisone, progesterone, testosterone; alfentanyl, aripiprazole, buspirone, cafergot, caffeine, cilostazol, cocaine, codeine, dapsone, dextromethorphan, docetaxel, domperidone, eplerenone, fentanyl, finasteride, gleevec, haloperidol, irinotecan, Levo-Alpha Acetyl Methadol (LAAM), lidocaine, methadone, nateglinide, odanestron, pimozide, propranolol, quinine, salmeterol, sildenafil, sirolimus, tamoxifen, taxol, terfenadine, trazodone, vincristine, zaleplon, and zolpidem.

In any of the embodiments described herein, the substance can be an active agent having a narrow therapeutic index. Examples of narrow therapeutic index active agents include warfarin, phenytoin, fosphenytoin, thioridazine, theophylline, cyclosporine, and pimozide.

In some embodiments, the active agent comprises warfarin. Warfarin, 3-α-acetonylbenzyl)-4-hydroxycoumarin, is an anticoagulant, which is eliminated by metabolism by cytochrome p450 isoforms including CYP2C9, CYP2C19, CYP2C8, CYP2C18, CYP1A2, and CYP3A4. Warfarin has a narrow therapeutic index such that too little can lead to excessive clotting, while excessive warfarin can lead to excessive bleeding. The dosing of warfarin is individualized according to the patient's sensitivity to the active agent as indicated, for example, by the Prothrombin Time/International Normalized Ratio (PT/INR). The PT/INR gives an indication of how fast blood is clotting. The recommended initial dose is 2–5 mg/day, with 2–10 mg/day as the maintenance dose. Warfarin tablets for oral administration include tablets comprising 1, 2, 2.5, 3, 4, 5, 6, 7.5, and 10 mg of warfarin. The INR may be adjusted to 2.0–4.5, or 2.0–3.0 or 2.5–3.5 depending on whether the warfarin is being administered to treat venous thromboembolism, non-valvular atrial fibrillation, post-myocardial infarction, heart valve prophylaxis, or recurrent systemic embolism.

In the PT test, a reagent which induces coagulation is added to a sample of the patient's plasma. The reagent typically primarily comprises thromboplastin and calcium chloride. Many commercially available PT reagents contain crude thromboplastin extracted from natural sources, e.g., rabbit brain, rabbit brain/lung mixtures, human placenta, or bovine brain, although recombinant thromboplastin may also be employed. Prothrombin time assays are performed by mixing the plasma sample and reagent at a constant temperature such as 37° C., and monitoring the progress of the reaction until a perceptible clot (or "gel clot") is detected. The development of a gel clot is the end point of the reaction. This end point may be detected in various ways such as by viscosity change, by electrode reaction, and, most commonly, by photometric means. The test result is generally compared to a result using a normal (control) plasma and converted to an INR.

The International Normalized Ratio, or INR, was developed to standardize PT values, so that test results from different thromboplastins and coagulation analyzers become equivalent. Under the INR system, a thromboplastin is assigned an International Sensitivity Index (ISI) value. The ISI indicates the relative sensitivity of the thromboplastin compared to an international reference thromboplastin. If a thromboplastin has the same sensitivity as the reference thromboplastin, then its ISI is 1.0. A higher ISI value indicates that a thromboplastin is less sensitive than the reference thromboplastin. The ISI is used in the following formula to calculate an INR value from a PT value: $INR=(\text{patient PT/mean normal PT})^{ISI}$. The ISI is usually determined by the thromboplastin manufacturer. Different ISI values are assigned for different models or classes of coagulation analyzers.

In another embodiment, the active agent comprises phenytoin. Phenytoin, 5,5-diphenylhydantoin, is an antiepileptic active agent useful in the treatment of epilepsy which is eliminated by metabolism by cytochrome p450 isoforms including CYP1A2, CYP2C9, CYP2C19, and CYP3A4. Phenytoin has a narrow therapeutic index such that too little can lead to insufficient results and excessive phenytoin can lead to phenytoin toxicity. The typical clinically effective serum level is about 10 to about 20 μg/mL. The recommended initial dose is one 100 mg capsule 3 to 4 times per day, with 300 mg/day dose in three divided doses or one single dose per day. The dosing of phenytoin can be individualized according to the patient's sensitivity to the active agent by measuring plasma concentration of phenytoin.

Methods of treating a musculoskeletal condition or head pain with metaxalone are provided herein. Such methods include informing a user that metaxalone affects the activity of a cytochrome p450 isozyme. The method may further include informing the user that administration of metaxalone with a substance can affect the plasma concentration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. The method may also include informing the user of any information disclosed herein about the effect of metaxalone or the substance on the activity of a cytochrome p450 isozyme and any information disclosed herein about the effect of metaxalone or the substance on the plasma con-

centration, bioavailability, safety, efficacy, or a combination comprising at least one of the foregoing of metaxalone or the substance. Methods of treatment may also include providing a user with metaxalone or administering metaxalone to a patient.

Methods of treatment include methods in which the user is a patient and additionally comprising administering metaxalone and an active agent to the patient. The patient may be, for example, a human patient, a patient in need of treatment of a musculoskeletal condition or head pain, a patient receiving prophylactic metaxalone treatment, or a patient undergoing metaxalone therapy. The amount of metaxalone administered may be a therapeutically effective amount.

Methods of treatment may additionally include monitoring the patient's plasma concentration of the active agent as $AUC_{0\text{-}INF}$, $AUC_{0\text{-}t}$, $C_{MAX}$, or a combination of any of the foregoing pharmacokinetic parameters. When metaxalone is administered together with another active agent, methods of treatment can include determining the plasma concentration of the active agent and altering dosing of the active agent for the patient based on the determined active agent plasma concentration.

In another embodiment, a method of treatment comprises administering to a patient in need of both a skeletal muscle relaxant and an anticoagulant, for example, metaxalone and warfarin, and monitoring the Prothrombin Time/International Normalized Ratio. Monitoring the Prothrombin Time/International Normalized Ratio may be performed for example daily, every other day, weekly, every other week, monthly, or according to another schedule or time criteria. The method may further comprise providing to the patient or medical care worker instructions regarding measuring the Prothrombin Time/International Normalized Ratio.

When the substance administered with metaxalone is an NTI active agent, methods using a blood test to monitor plasma levels of the NTI active agent comprise administering to a patient metaxalone and the NTI active agent, and monitoring the blood levels of the NTI active agent as $AUC_{0\text{-}t}$, $AUC_{0\text{-}INF}$, $C_{MAX}$, or a combination comprising one or more of the foregoing pharmacokinetic parameters.

In one embodiment, a method of using a blood test to monitor warfarin levels comprises administering to a patient in need of both a skeletal muscle relaxant and an anticoagulant both metaxalone and warfarin, and monitoring the blood levels of warfarin as $AUC_{0\text{-}t}$, $AUC_{0\text{-}INF}$, $C_{MAX}$, or a combination comprising one or more of the foregoing pharmacokinetic parameters.

In another embodiment, the substance is phenytoin, and a method using a blood test to monitor plasma levels of phenytoin comprise administering to a patient metaxalone and phenytoin, and monitoring the blood levels of phenytoin as $AUC_{0\text{-}t}$, $AUC_{0\text{-}INF}$, $C_{MAX}$, or a combination comprising one or more of the foregoing pharmacokinetic parameters.

In all of the embodiments herein, a medical care worker can determine the plasma concentration of an active agent by performing or ordering the performance of any suitable method. For example, the medical care worker could order a test using blood drawn from the patient for determining the plasma concentration of the active agent.

The invention is further illustrated by the following examples.

## EXAMPLE 1

### Determination of Human Cytochrome p450 Isozymes Using Metaxalone as a Substrate

The study of this example was performed to determine the metabolism of metaxalone by human cytochrome p450 isoforms CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Microsomes containing singly-expressed human CYP isoforms were incubated in the presence of metaxalone. The metabolism of metaxalone was evaluated by measuring the disappearance of metaxalone by high-performance liquid chromatography (HPLC).

Commercially available microsomes from baculovirus-infected insect cells containing singly-expressed recombinant wild-type (*1 allele) human CYP enzymes and cDNA-expressed human cytochrome p450 oxidoreductase [BD SUPERSOMES Enzymes; BD Biosciences Discovery Labware (Woburn, Mass.)] were used. For CYP2A6, CYP2C9, CYP2C19, and CYP2E1, the SUPERSOMES also expressed human cytochrome b5 in addition to human cytochrome p450 oxidoreductase and the human CYP isozyme.

Metaxalone stock solutions were prepared in methanol at 100 times (100x) the final concentration. The stock solutions were added to incubation mixtures to obtain final concentrations of 0.5, 2.5, and 25 μM (corresponding to 111, 552, and 5530 ng metaxalone/mL, respectively), each containing 1% methanol. All incubations were conducted at 37±1° C. in a shaking water bath with a sample size of N=3 replicates for each experimental group. Incubation mixtures of microsomes (corresponding to 10 pmol p450) and metaxalone were prepared in 0.1 M Tris buffer. After a 5-minute pre-incubation, an NADPH regenerating system (NRS) was added to the incubation mixtures to initiate reactions, with a final incubation volume of 0.5 mL. Incubations were continued for 30 minutes, and then terminated, except for those for CYP2C19, which were incubated for 36 minutes prior to termination. Samples were then analyzed for metaxalone.

Positive controls with a suitable isoform-selective substrate were performed for each CYP isoform to verify metabolic activity. Concentration of metabolites formed from CYP isoform-selective substrates in the positive control samples was analyzed using liquid chromatography/mass spectrometry (LC/MS) or HPLC, as appropriate. A table of the substrate, substrate concentration, solvent, metabolite formed, and metabolite assay method for each CYP isozyme studied is below.

| CYP isoform | Isoform-selective substrate | Substrate concentration | Solvent | Metabolite formed | Metabolite Assay |
|---|---|---|---|---|---|
| CYP1A2 | Phenacetin | 50 μM | ACN | Acetaminophen | LC/MS |
| CYP2A6 | Coumarin | 8 μM | ACN | 7-hydroxy coumarin | HPLC-UV |
| CYP2C9 | Tolbutamide | 150 μM | ACN | 4'-methylhydroxytolbutamide | LC/MS |
| CYP2C19 | S-Mephenytoin | 50 μM | ACN | 4'-hydroxy mephenytoin | LC/MS |
| CYP2D6 | Dextromethorphan | 5 μM | Water | dextrorphan | LC/MS |

-continued

| CYP isoform | Isoform-selective substrate | Substrate concentration | Solvent | Metabolite formed | Metabolite Assay |
|---|---|---|---|---|---|
| CYP2E1 | Chlorzoxazone | 50 μM | ACN | 6-hydroxy chlorzoxazone | LC/MS |
| CYP3A4 | Testosterone | 100 μM | ACN | 6β-hydroxy testosterone | HPLC-UV |

Matrix controls were performed to determine the background signal from the matrix components (microsomes (10 pmol p450), 0.1 N Tris buffer, NRS, and 1% methanol). Additionally metabolic negative controls were performed to distinguish potential nonenzymatic metabolism of metaxalone from p450-mediated metabolism. Incubation mixtures were prepared in 0.1 M Tris buffer with SUPERSOMES (10 pmol P450) and metaxalone (at each concentration). After a 5-minute pre-incubation, 2% sodium bicarbonate solution was added to the incubation mixtures. Incubation was for 30 minutes at a final volume of 0.5 mL. Matrix and metabolic negative controls were terminated by adding an equal volume of methanol. The matrix control and metabolic negative control samples were analyzed for metaxalone by HPLC. Analysis of samples was subsequent to storage at −70° C.

Results are presented for each studied human cytochrome p450 isozyme in Tables 2–8.

TABLE 2

Metabolism of Metaxalone by Expressed Recombinant Human CYP1A2

| Metaxalone Concentration (μM) | Metaxalone Present Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Percent of Metabolic Negative Control Individual | Percent of Metabolic Negative Control Mean ± SD |
|---|---|---|---|---|---|
| MNC | 0.20195 | 0.404 | 0.391 ± 0.0113 | 103 | 100 ± 2.88 |
| (0.5) | 0.19430 | 0.389 | | 99.3 | |
| | 0.19097 | 0.382 | | 97.6 | |
| 0.5 | 0.15087 | 0.302 | 0.352 ± 0.0761 | 77.1 | 89.9 ± 19.4 |
| | 0.21975 | 0.440 | | 112 | |
| | 0.15734 | 0.315 | | 80.4 | |
| MNC | 0.65183 | 1.30 | 1.33 ± 0.0221 | 98.3 | 100 ± 1.67 |
| (2.5) | 0.66350 | 1.33 | | 100 | |
| | 0.67394 | 1.35 | | 102 | |
| 2.5 | 0.52700 | 1.05 | 1.07 ± 0.0167 | 79.5 | 80.4 ± 1.26 |
| | 0.52908 | 1.06 | | 79.8 | |
| | 0.54235 | 1.08 | | 81.8 | |
| MNC | 10.11453 | 20.2 | 19.8 ± 0.360 | 102 | 100 ± 1.82 |
| (25) | 9.76568 | 19.5 | | 98.5 | |
| | 9.86156 | 19.7 | | 99.5 | |
| 25 | 8.20521 | 16.4 | 16.6 ± 0.337 | 82.8 | 83.7 ± 1.70 |
| | 8.19232 | 16.4 | | 82.6 | |
| | 8.49030 | 17.0 | | 85.6 | |
| MXC | 0.00000* | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000* | N/A | | N/A | |
| | 0.00000* | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable
*The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM).
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 3

Metabolism of Metaxalone by Expressed Recombinant Human CYP2A6

| Metaxalone Concentration (μM) | Metaxalone Present Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Percent of Metabolic Negative Control Individual | Percent of Metabolic Negative Control Mean ± SD |
|---|---|---|---|---|---|
| MNC | 0.15455 | 0.309 | 0.311 ± 0.00446 | 99.4 | 100 ± 1.43 |
| (0.5) | 0.15795 | 0.316 | | 102 | |
| | 0.15375 | 0.308 | | 98.9 | |
| 0.5 | 0.15457 | 0.309 | 0.299 ± 0.0124 | 99.5 | 96.1 ± 3.99 |
| | 0.15112 | 0.302 | | 97.2 | |
| | 0.14253 | 0.285 | | 91.7 | |
| MNC | 0.74261 | 1.49 | 1.52 ± 0.0353 | 97.9 | 100 ± 2.33 |

TABLE 3-continued

Metabolism of Metaxalone by Expressed Recombinant Human CYP2A6

| Metaxalone | Metaxalone Present | | | Percent of Metabolic | |
|---|---|---|---|---|---|
| Concentration | Raw | Adjusted (μM) | | Negative Control | |
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD |
| (2.5) | 0.75568 | 1.51 | | 99.6 | |
| | 0.77755 | 1.56 | | 102 | |
| 2.5 | 0.79130 | 1.58 | 1.61 ± 0.0373 | 104 | 106 ± 2.46 |
| | 0.79791 | 1.60 | | 105 | |
| | 0.82642 | 1.65 | | 109 | |
| MNC | 7.74594 | 15.5 | 15.3 ± 0.147 | 101 | 100 ± 0.959 |
| (25) | 7.64948 | 15.3 | | 99.8 | |
| | 7.60163 | 15.2 | | 99.2 | |
| 25 | 7.76399 | 15.5 | 15.6 ± 0.0975 | 101 | 102 ± 0.636 |
| | 7.85044 | 15.7 | | 102 | |
| | 7.84628 | 15.7 | | 102 | |
| MXC | 0.00000* | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000* | N/A | | N/A | |
| | 0.00000* | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable

*The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 4

Metabolism of Metaxalone by Expressed Recombinant Human CYP2C9

| Metaxalone | Metaxalone Present | | | Percent of Metabolic | |
|---|---|---|---|---|---|
| Concentration | Raw | Adjusted (μM) | | Negative Control | |
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD |
| MNC | 0.17052 | 0.341 | 0.348 ± 0.00997 | 97.9 | 100 ± 2.86 |
| (0.5) | 0.17229 | 0.345 | | 98.9 | |
| | 0.17990 | 0.360 | | 103 | |
| 0.5 | 0.18004 | 0.360 | 0.355 ± 0.00608 | 103 | 102 ± 1.75 |
| | 0.17784 | 0.356 | | 102 | |
| | 0.17403 | 0.348 | | 99.9 | |
| MNC | 0.93197 | 1.86 | 1.93 ± 0.0605 | 96.8 | 100 ± 3.14 |
| (2.5) | 0.96526 | 1.93 | | 100 | |
| | 0.99235 | 1.98 | | 103 | |
| 2.5 | 0.96842 | 1.94 | 1.92 ± 0.0246 | 101 | 99.7 ± 1.28 |
| | 0.96593 | 1.93 | | 100 | |
| | 0.94597 | 1.89 | | 98.2 | |
| MNC | 10.31249 | 20.6 | 21.3 ± 0.620 | 97.1 | 100 ± 2.92 |
| (25) | 10.63201 | 21.3 | | 100 | |
| | 10.93245 | 21.9 | | 103 | |
| 25 | 10.66111 | 21.3 | 21.5 ± 0.144 | 100 | 101 ± 0.675 |
| | 10.80454 | 21.6 | | 102 | |
| | 10.72836 | 21.5 | | 101 | |
| MXC | 0.00000* | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000* | N/A | | N/A | |
| | 0.00000* | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable

*The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 5

Metabolism of Metaxalone by Expressed Recombinant Human CYP2C19

| Metaxalone Concentration (μM) | Metaxalone Present Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Percent of Metabolic Negative Control Individual | Percent of Metabolic Negative Control Mean ± SD |
|---|---|---|---|---|---|
| MNC | 0.18718 | 0.374 | 0.370 ± 0.00898 | 101 | 100 ± 2.43 |
| (0.5) | 0.18763 | 0.375 | | 102 | |
| | 0.17964 | 0.359 | | 97.2 | |
| 0.5 | 0.16773 | 0.335 | 0.345 ± 0.0104 | 90.8 | 93.4 ± 2.82 |
| | 0.17180 | 0.344 | | 93.0 | |
| | 0.17808 | 0.356 | | 96.4 | |
| MNC | 0.72720 | 1.45 | 1.39 ± 0.0560 | 105 | 100 ± 4.03 |
| (2.5) | 0.67562 | 1.35 | | 97.2 | |
| | 0.68261 | 1.37 | | 98.2 | |
| 2.5 | 0.67218 | 1.34 | 1.34 ± 0.00561 | 96.7 | 96.5 ± 0.404 |
| | 0.67254 | 1.35 | | 96.7 | |
| | 0.66751 | 1.34 | | 96.0 | |
| MNC | 9.84488 | 19.7 | 20.1 ± 1.03 | 97.8 | 100 ± 5.13 |
| (25) | 9.69255 | 19.4 | | 96.3 | |
| | 10.65287 | 21.3 | | 106 | |
| 25 | 9.34508 | 18.7 | 18.6 ± 0.120 | 92.9 | 92.6 ± 0.597 |
| | 9.35948 | 18.7 | | 93.0 | |
| | 9.24903 | 18.5 | | 91.9 | |
| MXC | 0.00000[a] | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000[a] | N/A | | N/A | |
| | 0.06454 | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable

[a]The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 6

Metabolism of Metaxalone by Expressed Recombinant Human CYP2D6

| Metaxalone Concentration (μM) | Metaxalone Present Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Percent of Metabolic Negative Control Individual | Percent of Metabolic Negative Control Mean ± SD |
|---|---|---|---|---|---|
| MNC | 0.14509 | 0.290 | 0.292 ± 0.00220 | 99.4 | 100 ± 0.755 |
| (0.5) | 0.14716 | 0.294 | | 101 | |
| | 0.14547 | 0.291 | | 99.7 | |
| 0.5 | 0.18683 | 0.374 | 0.319 ± 0.0477 | 128 | 109 ± 16.3 |
| | 0.14857 | 0.297 | | 102 | |
| | 0.14305 | 0.286 | | 98.0 | |
| MNC | 0.79025 | 1.58 | 1.56 ± 0.0184 | 101 | 100 ± 1.18 |
| (2.5) | 0.78433 | 1.57 | | 100 | |
| | 0.77221 | 1.54 | | 98.7 | |
| 2.5 | 0.75826 | 1.52 | 1.53 ± 0.0111 | 96.9 | 97.7 ± 0.707 |
| | 0.76852 | 1.54 | | 98.2 | |
| | 0.76697 | 1.53 | | 98.0 | |
| MNC | 9.63762 | 19.3 | 19.2 ± 0.0994 | 100 | 100 ± 0.517 |
| (25) | 9.54788 | 19.1 | | 99.4 | |
| | 9.62976 | 19.3 | | 100 | |
| 25 | 9.52577 | 19.1 | 19.2 ± 0.436 | 99.2 | 99.9 ± 2.27 |
| | 9.84529 | 19.7 | | 103 | |
| | 9.42917 | 18.9 | | 98.2 | |
| MXC | 0.00000[a] | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000[a] | N/A | | N/A | |
| | 0.00000[a] | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable

[a]The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 7

Metabolism of Metaxalone by Expressed Recombinant Human CYP2E1

| Metaxalone Concentration (μM) | Metaxalone Present: Raw (μM) | Adjusted (μM): Individual | Adjusted (μM): Mean ± SD | Percent of Metabolic Negative Control: Individual | Percent of Metabolic Negative Control: Mean ± SD |
|---|---|---|---|---|---|
| MNC | 0.18358 | 0.367 | 0.355 ± 0.0104 | 103 | 100 ± 2.92 |
| (0.5) | 0.17510 | 0.350 | | 98.6 | |
| | 0.17416 | 0.348 | | 98.1 | |
| 0.5 | 0.17871 | 0.357 | 0.352 ± 0.00648 | 101 | 99.0 ± 1.83 |
| | 0.17235 | 0.345 | | 97.0 | |
| | 0.17662 | 0.353 | | 99.4 | |
| MNC | 0.89075 | 1.78 | 1.69 ± 0.117 | 105 | 100 ± 6.89 |
| (2.5) | 0.77998 | 1.56 | | 92.2 | |
| | 0.86695 | 1.73 | | 102 | |
| 2.5 | 0.88299 | 1.77 | 1.76 ± 0.00318 | 104 | 104 ± 0.188 |
| | 0.87990 | 1.76 | | 104 | |
| | 0.88209 | 1.76 | | 104 | |
| MNC | 9.11125 | 18.2 | 17.8 ± 0.410 | 103 | 100 ± 2.30 |
| (25) | 8.70811 | 17.4 | | 98.0 | |
| | 8.84728 | 17.7 | | 99.5 | |
| 25 | 8.73183 | 17.5 | 19.2 ± 2.71 | 98.2 | 108 ± 15.3 |
| | 11.15149 | 22.3 | | 125 | |
| | 8.87878 | 17.8 | | 99.9 | |
| MXC | 0.00000[a] | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000[a] | N/A | | N/A | |
| | 0.00000[a] | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable

[a]The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 8

Metabolism of Metaxalone by Expressed Recombinant Human CYP3A4

| Metaxalone Concentration (μM) | Metaxalone Present: Raw (μM) | Adjusted (μM): Individual | Adjusted (μM): Mean ± SD | Percent of Metabolic Negative Control: Individual | Percent of Metabolic Negative Control: Mean ± SD |
|---|---|---|---|---|---|
| MNC | 0.16014 | 0.320 | 0.318 ± 0.00502 | 101 | 100 ± 1.58 |
| (0.5) | 0.15592 | 0.312 | | 98.2 | |
| | 0.16039 | 0.321 | | 101 | |
| 0.5 | 0.15978 | 0.320 | 0.320 ± 0.00333 | 101 | 101 ± 1.05 |
| | 0.16159 | 0.323 | | 102 | |
| | 0.15826 | 0.317 | | 99.6 | |
| MNC | 0.85285 | 1.71 | 1.72 ± 0.127 | 99.3 | 100 ± 0.741 |
| (2.5) | 0.86553 | 1.73 | | 101 | |
| | 0.85828 | 1.72 | | 99.9 | |
| 2.5 | 0.85730 | 1.71 | 1.68 ± 0.0289 | 99.8 | 98.0 ± 0.168 |
| | 0.82923 | 1.66 | | 96.5 | |
| | 0.83738 | 1.67 | | 97.5 | |
| MNC | 8.65154 | 17.3 | 17.4 ± 0.0906 | 99.4 | 100 ± 0.521 |
| (25) | 8.71767 | 17.4 | | 100 | |
| | 8.73830 | 17.5 | | 100 | |
| 25 | 8.53809 | 17.1 | 17.1 ± 0.192 | 98.1 | 98.1 ± 1.10 |
| | 8.44686 | 16.9 | | 97.1 | |
| | 8.63905 | 17.3 | | 99.3 | |
| MXC | 0.00000[a] | N/A | N/A ± N/A | N/A | N/A ± N/A |
| (0) | 0.00000[a] | N/A | | N/A | |
| | 0.00000[a] | N/A | | N/A | |

Abbreviations: SD, standard deviation; MNC, metabolic negative control; MXC, matrix control; N/A, not applicable

[a]The Raw value (μM) was below the lowest concentration on the standard curve (0.05 μM)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Tables 2 and 5 show the results for human CYP1A2 and CYP2C19, respectively. The results for these two cytochrome p450 isozymes show that metaxalone is a substrate for the enzymatic activity of both CYP 1A2 and CYP2C19.

Disappearance of metaxalone was detected following incubation with CYP1A2 in the presence of the NADPH-regenerating system. Disappearance of metaxalone ranged from 10.1% to 19.6% (Table 2). The difference from the starting amount is statistically significant at 2.5 and 25 μM using an unpaired two-tailed t-test ($p \leqq 0.05$). These results indicate that CYP1A2 is involved in the metabolism of metaxalone.

In the experiments with CYP2C19, metaxalone disappearance was evident following incubation with metaxalone at all three concentrations (Table 5). The mean disappearance of metaxalone was 6.6% for the reaction using 0.5 μM metaxalone; the reduction in the mean amount of metaxalone from the value for the corresponding metabolic negative control was statistically significant ($p \leqq 0.05$) using an unpaired two-tailed t-test. The amount of the disappearance of metaxalone observed at 2.5 or 25 μM was not statistically significant ($p > 0.05$) compared to the mean values for the corresponding metabolic negative controls using a two-tailed t-test. These results indicate that CYP2C19 is also involved in the metabolism of metaxalone, though to a lesser extent than CYP1A2.

Experiments with the other tested cytochrome p450 isozymes (Tables 3–4 and 6–8) failed to show any statistically significant disappearance of metaxalone following incubation at the standard conditions, indicating that, within the limits of detection for these experiments, metaxalone was not used as a substrate by the other tested cytochrome p450 isozymes: CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4.

## EXAMPLE 2

### Metaxalone Inhibition of Cytochrome p450 Isozymes in Human Microsomes

The study of this example was performed to determine the potential of metaxalone to inhibit the activities of cytochrome p450 (CYP) isoforms CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes. Human liver microsomes were incubated in the presence of metaxalone and a substrate selective for each CYP isoform. A table of the substrate, substrate concentration, solvent, metabolite formed and metabolite assay method for each CYP isozyme studied is below.

Metaxalone stock solutions were prepared in methanol at 100 times (100x) the final concentration and added to incubation mixtures to obtain final concentrations of 0.3, 1, 3, 30, and 100 μM (corresponding to 66.3, 221, 663, 6630 and 22,100 ng metaxalone/mL, respectively), each containing 1% methanol.

Microsomes were prepared by differential centrifugation of liver homogenates pooled from at least ten human donors.

All metaxalone incubations were conducted at 37±1° C. in a shaking water bath using a sample size of N=3 replicates for experimental groups. Incubation mixtures were prepared in 0.1 M Tris buffer and contained microsomes (0.25 mg protein/mL for CYP2C9, CYP2D6, CYP2E1, and CYP3A4; 0.5 mg protein/mL for CYP1A2, CYP2A6, CYP2B6, CYP2C8, and CYP2C19), metaxalone (at each concentration), and a CYP isoform-selective substrate. After a 5 minute preincubation, NADPH regenerating system (NRS) was added to initiate the reaction. CYP2A6 and CYP3A4 incubations were for 10 minutes. All other incubations were for 30 minutes.

Incubations for CYP2C8 were terminated by adding 1.0 mL of ACN, while all other incubations were terminated by adding 1.0 mL of methanol. Samples were transferred to cryovials and analyzed after storage at −70° C. Triplicate replicates were performed for each concentration of metaxalone for each cytochrome p450 isozyme.

To verify that the test system was responsive to inhibitors, a positive control using 1 μM ketoconazole, a selective inhibitor of CYP3A4, was added to CYP3A4 microsome incubations with 100 μM testosterone. Four replicates were performed. The test system was considered responsive to inhibitors since the mean specific activity of CYP3A4 in the positive control samples treated with ketoconazole was <14% of the mean specific activity in the corresponding vehicle control samples.

Vehicle control experiments were performed to establish a baseline value for enzyme activity. Incubation mixtures were prepared in 0.1 M Tris buffer with microsomes (0.25 mg protein/mL for CYP2C9, CYP2D6, CYP2E1, and CYP3A4; 0.5 mg protein/mL for CYP1A2, CYP2A6, CYP2B6, CYP2C8, and CYP2C19), 1% methanol, and a CYP isoform-selective substrate. Four replicates were performed.

Metaxalone interference control samples were also included to eliminate the possibility of interference by metaxalone or its metabolites in detection of the metabolite formed from an isoform-selective substrate. Two replicates were performed. Incubation mixtures containing microsomes (0.25 mg protein/mL for CYP2C9, CYP2D6, CYP2E1, and CYP3A4; 0.5 mg protein/mL for CYP1A2, CYP2A6, CYP2B6, CYP2C8, and CYP2C19), 100 μM metaxalone, and 1% substrate solvent were prepared in 0.1

| CYP isoform | Isoform-selective substrate | Substrate concentration | Solvent | Metabolite formed | Metabolite Assay |
|---|---|---|---|---|---|
| CYP1A2 | Phenacetin | 50 μM | ACN | acetaminophen | LC/MS |
| CYP2A6 | Coumarin | 8 μM | ACN | 7-hydroxy coumarin | HPLC-UV |
| CYP2B6 | S-Mephenytoin | 1 mM | ACN | nirvanol | LC/MS |
| CYP2C8 | Paclitaxel | 5 μM | ACN | 6-hydroxy paclitaxel | LC/MS |
| CYP2C9 | Tolbutamide | 150 μM | ACN | 4'-methylhydroxytolbutamide | LC/MS |
| CYP2C19 | S-Mephenytoin | 50 μM | ACN | 4'-hydroxy mephenytoin | LC/MS |
| CYP2D6 | Dextromethorphan | 5 μM | Water | dextrorphan | LC/MS |
| CYP2E1 | Chlorzoxazone | 50 μM | ACN | 6-hydroxy chlorzoxazone | LC/MS |
| CYP3A4 | Testosterone | 100 μM | ACN | 6β-hydroxy testosterone | HPLC-UV |

M Tris buffer. No interference was detected in any of the metabolite assay methods used.

Results for each CYP isoform, in the presence and absence of metaxalone, are reported in Tables 9–17.

TABLE 9

CYP1A2 Activity in Pooled Human Microsomes

| | Acetaminophen formation | | | Specific Activity | | |
|---|---|---|---|---|---|---|
| Metaxalone | Raw | Adjusted (μM) | | (pmol/min/mg protein) | | Percent |
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD | of VC |
| 0 | 0.23653 | 0.237 | 0.216 ± 0.0138 | 31.5 | 28.8 ± 1.84 | 100 |
| (VC) | 0.21124 | 0.211 | | 28.2 | | |
| | 0.21156 | 0.212 | | 28.2 | | |
| | 0.20568 | 0.206 | | 27.4 | | |
| 0.3 | 0.21120 | 0.211 | 0.210 ± 0.00536 | 28.2 | 28.0 ± 0.715 | 97.2 |
| | 0.21487 | 0.215 | | 28.6 | | |
| | 0.20431 | 0.204 | | 27.2 | | |
| 1 | 0.19966 | 0.200 | 0.200 ± 0.00246 | 26.6 | 26.6 ± 0.327 | 92.3 |
| | 0.19709 | 0.197 | | 26.3 | | |
| | 0.20200 | 0.202 | | 26.9 | | |
| 3 | 0.19900 | 0.199 | 0.195 ± 0.00589 | 26.5 | 26.0 ± 0.785 | 90.3 |
| | 0.18839 | 0.188 | | 25.1 | | |
| | 0.19813 | 0.198 | | 26.4 | | |
| 30 | 0.18924 | 0.189 | 0.194 ± 0.00544 | 25.2 | 25.9 ± 0.725 | 89.8 |
| | 0.19323 | 0.193 | | 25.8 | | |
| | 0.20000 | 0.200 | | 26.7 | | |
| 100 | 0.17757 | 0.178 | 0.177 ± 0.000206 | 23.7 | 23.6 ± 0.0275 | 82.0 |
| | 0.17733 | 0.177 | | 23.6 | | |
| | 0.17716 | 0.177 | | 23.6 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol).

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 10

CYP2A6 Activity in Pooled Human Microsomes

| | 7-Hydroxycoumarin formation | | | Specific Activity | | |
|---|---|---|---|---|---|---|
| Metaxalone | Raw | Adjusted (μM) | | (pmol/min/mg protein) | | Percent |
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD | of VC |
| 0 | 1.03214 | 1.03 | 1.06 ± 0.0356 | 413 | 426 ± 14.2 | 100 |
| (VC) | 1.04464 | 1.04 | | 418 | | |
| | 1.06891 | 1.07 | | 428 | | |
| | 1.11282 | 1.11 | | 445 | | |
| 0.3 | 1.07439 | 1.07 | 1.03 ± 0.0399 | 430 | 413 ± 16.0 | 96.9 |
| | 0.99553 | 0.996 | | 398 | | |
| | 1.02457 | 1.02 | | 410 | | |
| 1 | 0.99854 | 0.999 | 1.02 ± 0.0184 | 399 | 407 ± 7.36 | 95.7 |
| | 1.02269 | 1.02 | | 409 | | |
| | 1.03468 | 1.03 | | 414 | | |
| 3 | 1.05100 | 1.05 | 1.09 ± 0.0402 | 420 | 436 ± 16.1 | 102 |
| | 1.13132 | 1.13 | | 453 | | |
| | 1.08822 | 1.09 | | 435 | | |
| 30 | 1.08205 | 1.08 | 1.14 ± 0.0493 | 433 | 455 ± 19.7 | 107 |
| | 1.15129 | 1.15 | | 461 | | |
| | 1.17736 | 1.18 | | 471 | | |
| 100 | 0.98864 | 0.989 | 1.01 ± 0.0416 | 395 | 404 ± 16.6 | 94.8 |
| | 0.98209 | 0.982 | | 393 | | |
| | 1.05713 | 1.06 | | 423 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 11

CYP2B6 Activity in Pooled Human Microsomes

| Metaxalone (μM) | Nirvanol formation Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 0 | 0.23500 | 0.235 | 0.225 ± 0.0120 | 31.3 | 29.9 ± 1.60 | 100 |
| (VC) | 0.23266 | 0.233 | | 31.0 | | |
| | 0.22199 | 0.222 | | 29.6 | | |
| | 0.20877 | 0.209 | | 27.8 | | |
| 0.3 | 0.20942 | 0.209 | 0.203 ± 0.00904 | 27.9 | 27.0 ± 1.21 | 90.2 |
| | 0.19234 | 0.192 | | 25.6 | | |
| | 0.20601 | 0.206 | | 27.5 | | |
| 1 | 0.20438 | 0.204 | 0.223 ± 0.0201 | 27.3 | 29.8 ± 2.68 | 99.5 |
| | 0.22144 | 0.221 | | 29.5 | | |
| | 0.24442 | 0.244 | | 32.6 | | |
| 3 | 0.19695 | 0.197 | 0.203 ± 0.00751 | 26.3 | 27.1 ± 1.00 | 90.6 |
| | 0.20166 | 0.202 | | 26.9 | | |
| | 0.21166 | 0.212 | | 28.2 | | |
| 30 | 0.21681 | 0.217 | 0.217 ± 0.00162 | 28.9 | 28.9 ± 0.216 | 96.6 |
| | 0.21548 | 0.215 | | 28.7 | | |
| | 0.21871 | 0.219 | | 29.2 | | |
| 100 | 0.18648 | 0.186 | 0.188 ± 0.00436 | 24.9 | 25.1 ± 0.581 | 83.7 |
| | 0.18463 | 0.185 | | 24.6 | | |
| | 0.19293 | 0.193 | | 25.7 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 12

CYP2C8 Activity in Pooled Human Microsomes

| Metaxalone (μM) | 6-Hydroxypaclitaxel formation Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 0 | 0.13462 | 0.135 | 0.136 ± 0.00522 | 17.9 | 18.2 ± 0.696 | 100 |
| (VC) | 0.14017 | 0.140 | | 18.7 | | |
| | 0.14074 | 0.141 | | 18.8 | | |
| | 0.12965 | 0.130 | | 17.3 | | |
| 0.3 | 0.14476 | 0.145 | 0.126 ± 0.0163 | 19.3 | 16.8 ± 2.18 | 92.7 |
| | 0.11377 | 0.114 | | 15.2 | | |
| | 0.12042 | 0.120 | | 16.1 | | |
| 1 | 0.13927 | 0.139 | 0.140 ± 0.00305 | 18.6 | 18.7 ± 0.406 | 103 |
| | 0.13749 | 0.137 | | 18.3 | | |
| | 0.14343 | 0.143 | | 19.1 | | |
| 3 | 0.15034 | 0.150 | 0.149 ± 0.00174 | 20.0 | 19.9 ± 0.232 | 109 |
| | 0.14945 | 0.149 | | 19.9 | | |
| | 0.14698 | 0.147 | | 19.6 | | |
| 30 | 0.14949 | 0.149 | 0.138 ± 0.0114 | 19.9 | 18.4 ± 1.52 | 101 |
| | 0.13724 | 0.137 | | 18.3 | | |
| | 0.12667 | 0.127 | | 16.9 | | |
| 100 | 0.13170 | 0.132 | 0.133 ± 0.0207 | 17.6 | 17.8 ± 2.76 | 97.8 |
| | 0.15467 | 0.155 | | 20.6 | | |
| | 0.11340 | 0.113 | | 15.1 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 13

CYP2C9 Activity in Pooled Human Microsomes

| | 4'-Methylhydroxytolbutamide formation | | | Specific Activity | | |
|---|---|---|---|---|---|---|
| Metaxalone | Raw | Adjusted (μM) | | (pmol/min/mg protein) | | Percent |
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD | of VC |
| 0 | 0.17476 | 0.175 | 0.166 ± 0.0208 | 46.6 | 44.3 ± 5.54 | 100 |
| (VC) | 0.14904 | 0.149 | | 39.7 | | |
| | 0.14954 | 0.150 | | 39.9 | | |
| | 0.19164 | 0.192 | | 51.1 | | |
| 0.3 | 0.13620 | 0.136 | 0.135 ± 0.00106 | 36.3 | 36.1 ± 0.283 | 81.4 |
| | 0.13415 | 0.134 | | 35.8 | | |
| | 0.13565 | 0.136 | | 36.2 | | |
| 1 | 0.15107 | 0.151 | 0.136 ± 0.0187 | 40.3 | 36.2 ± 4.98 | 81.6 |
| | 0.14080 | 0.141 | | 37.5 | | |
| | 0.11485 | 0.115 | | 30.6 | | |
| 3 | 0.13051 | 0.131 | 0.135 ± 0.0103 | 34.8 | 36.0 ± 2.75 | 81.2 |
| | 0.12759 | 0.128 | | 34.0 | | |
| | 0.14670 | 0.147 | | 39.1 | | |
| 30 | 0.14975 | 0.150 | 0.151 ± 0.00841 | 39.9 | 40.3 ± 2.24 | 91.0 |
| | 0.14376 | 0.144 | | 38.3 | | |
| | 0.16037 | 0.160 | | 42.8 | | |
| 100 | 0.16269 | 0.163 | 0.145 ± 0.0150 | 43.4 | 38.8 ± 4.00 | 87.4 |
| | 0.13711 | 0.137 | | 36.6 | | |
| | 0.13627 | 0.136 | | 36.3 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 14

CYP2C19 Activity in Pooled Human Microsomes

| | 4'-Hydroxymephenytoin formation | | | Specific Activity | | |
|---|---|---|---|---|---|---|
| Metaxalone | Raw | Adjusted (μM) | | (pmol/min/mg protein) | | Percent |
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD | of VC |
| 0 | 0.16904 | 0.169 | 0.168 ± 0.00550 | 22.5 | 22.4 ± 0.733 | 100 |
| (VC) | 0.17373 | 0.174 | | 23.2 | | |
| | 0.16915 | 0.169 | | 22.6 | | |
| | 0.16055 | 0.161 | | 21.4 | | |
| 0.3 | 0.13971 | 0.140 | 0.142 ± 0.00299 | 18.6 | 19.0 ± 0.399 | 84.6 |
| | 0.14558 | 0.146 | | 19.4 | | |
| | 0.14164 | 0.142 | | 18.9 | | |
| 1 | 0.11367 | 0.114 | 0.113 ± 0.00140 | 15.2 | 15.0 ± 0.186 | 67.0 |
| | 0.11336 | 0.113 | | 15.1 | | |
| | 0.11111 | 0.111 | | 14.8 | | |
| 3 | 0.11597 | 0.116 | 0.114 ± 0.00238 | 15.5 | 15.2 ± 0.317 | 67.7 |
| | 0.11127 | 0.111 | | 14.8 | | |
| | 0.11423 | 0.114 | | 15.2 | | |
| 30 | 0.08336 | 0.0834 | 0.107 ± 0.0211 | 11.1 | 14.3 ± 2.82 | 63.8 |
| | 0.12339 | 0.123 | | 16.5 | | |
| | 0.11502 | 0.115 | | 15.3 | | |
| 100 | 0.10857 | 0.109 | 0.109 ± 0.00205 | 14.5 | 14.5 ± 0.274 | 64.9 |
| | 0.11132 | 0.111 | | 14.8 | | |
| | 0.10730 | 0.107 | | 14.3 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 15

CYP2D6 Activity in Pooled Human Microsomes

| Metaxalone | Dextrorphan formation Raw | Adjusted (μM) | | Specific Activity (pmol/min/mg protein) | | Percent |
|---|---|---|---|---|---|---|
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD | of VC |
| 0 | 0.18550 | 0.186 | 0.183 ± 0.00342 | 49.5 | 48.9 ± 0.911 | 100 |
| (VC) | 0.18569 | 0.186 | | 49.5 | | |
| | 0.18424 | 0.184 | | 49.1 | | |
| | 0.17843 | 0.178 | | 47.6 | | |
| 0.3 | 0.14820 | 0.148 | 0.149 ± 0.00258 | 39.5 | 39.8 ± 0.688 | 81.3 |
| | 0.14716 | 0.147 | | 39.2 | | |
| | 0.15206 | 0.152 | | 40.5 | | |
| 1 | 0.15910 | 0.159 | 0.154 ± 0.00482 | 42.4 | 41.2 ± 1.28 | 84.2 |
| | 0.14949 | 0.149 | | 39.9 | | |
| | 0.15485 | 0.155 | | 41.3 | | |
| 3 | 0.16116 | 0.161 | 0.164 ± 0.00353 | 43.0 | 43.7 ± 0.940 | 89.3 |
| | 0.16267 | 0.163 | | 43.4 | | |
| | 0.16788 | 0.168 | | 44.8 | | |
| 30 | 0.15533 | 0.155 | 0.156 ± 0.00335 | 41.4 | 41.6 ± 0.893 | 85.1 |
| | 0.15983 | 0.160 | | 42.6 | | |
| | 0.15328 | 0.153 | | 40.9 | | |
| 100 | 0.15992 | 0.160 | 0.158 ± 0.00255 | 42.6 | 42.0 ± 0.680 | 85.9 |
| | 0.15489 | 0.155 | | 41.3 | | |
| | 0.15813 | 0.158 | | 42.2 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 16

CYP2B1 Activity in Pooled Human Microsomes

| Metaxalone | 6-Hydroxychlorzoxazone formation Raw | Adjusted (μM) | | Specific Activity (pmol/min/mg protein) | | Percent |
|---|---|---|---|---|---|---|
| (μM) | (μM) | Individual | Mean ± SD | Individual | Mean ± SD | of VC |
| 0 | 0.85346 | 0.853 | 0.852 ± 0.0127 | 228 | 227 ± 3.39 | 100 |
| (VC) | 0.86925 | 0.869 | | 232 | | |
| | 0.84615 | 0.846 | | 226 | | |
| | 0.83969 | 0.840 | | 224 | | |
| 0.3 | 0.73634 | 0.736 | 0.710 ± 0.0228 | 196 | 189 ± 6.08 | 83.3 |
| | 0.69947 | 0.699 | | 187 | | |
| | 0.69469 | 0.695 | | 185 | | |
| 1 | 0.72701 | 0.727 | 0.716 ± 0.0194 | 194 | 191 ± 5.18 | 84.0 |
| | 0.72685 | 0.727 | | 194 | | |
| | 0.69326 | 0.693 | | 185 | | |
| 3 | 0.76089 | 0.761 | 0.755 ± 0.0110 | 203 | 201 ± 2.94 | 88.6 |
| | 0.74221 | 0.742 | | 198 | | |
| | 0.76169 | 0.762 | | 203 | | |
| 30 | 0.71716 | 0.717 | 0.733 ± 0.0145 | 191 | 196 ± 3.88 | 86.1 |
| | 0.74538 | 0.745 | | 199 | | |
| | 0.73733 | 0.737 | | 197 | | |
| 100 | 0.74969 | 0.750 | 0.743 ± 0.0175 | 200 | 198 ± 4.66 | 87.2 |
| | 0.75620 | 0.756 | | 202 | | |
| | 0.72321 | 0.723 | | 193 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 17

CYP3A4 Activity in Pooled Human Microsomes

| Metaxalone (μM) | 6β-Hydroxytestosterone formation Raw (μM) | Adjusted (μM) Individual | Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 0 | 0.12662* | N/A | 0.742 ± 0.00679 | N/A | 594 ± 5.43 | 100 |
| (VC) | 0.74589 | 0.746 | | 597 | | |
| | 0.74640 | 0.746 | | 597 | | |
| | 0.73440 | 0.734 | | 588 | | |
| 0.3 | 0.64318 | 0.643 | 0.647 ± 0.0130 | 515 | 517 ± 10.4 | 87.1 |
| | 0.66083 | 0.661 | | 529 | | |
| | 0.63550 | 0.636 | | 508 | | |
| 1 | 0.65762 | 0.658 | 0.654 ± 0.00353 | 526 | 523 ± 2.83 | 88.1 |
| | 0.65446 | 0.654 | | 524 | | |
| | 0.65057 | 0.651 | | 520 | | |
| 3 | 0.67154 | 0.672 | 0.668 ± 0.00420 | 537 | 534 ± 3.36 | 90.0 |
| | 0.66336 | 0.663 | | 531 | | |
| | 0.66907 | 0.669 | | 535 | | |
| 30 | 0.62513 | 0.625 | 0.633 ± 0.0370 | 500 | 506 ± 29.6 | 85.2 |
| | 0.67282 | 0.673 | | 538 | | |
| | 0.59996 | 0.600 | | 480 | | |
| 100 | 0.63960 | 0.640 | 0.596 ± 0.0454 | 512 | 477 ± 36.3 | 80.3 |
| | 0.59940 | 0.599 | | 480 | | |
| | 0.54904 | 0.549 | | 439 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
*Sample has been removed from all calculations due to the incorrect volume being added to the sample to stop the reaction.
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Under these experimental conditions, metaxalone inhibited activities of CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human liver microsomes at one or more of the tested metaxalone concentrations at a statistically significant level ($p \leq 0.05$ using an unpaired two-tailed t-test). The inhibition ranged from 12.8% (CYP2E1) to 35.1% (CYP2C19) at a metaxalone concentration of 100 μM.

Under these experimental conditions, no tested concentration of metaxalone inhibited activity of CYP2A6, CYP2C8, or CYP2C9 in human liver microsomes at a statistically significant level ($p > 0.05$ using an unpaired two-tailed t-test).

## EXAMPLE 3

### Metaxalone Induction/Inhibition of Cytochrome p450 Isozymes

The study of this example was performed to determine if there is induction or inhibition by metaxalone of cytochrome p450 isozymes CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. These induction/inhibition studies used cryopreserved human hepatocytes and compared enzymatic activity levels for each of these cytochrome p450 isozymes, using an appropriate enzyme substrate, in the human hepatocytes following in vitro exposure for 48±3 hrs in the presence or absence of metaxalone.

Hepatocytes from three human donors were obtained from a cryopreserved hepatocyte bank (In Vitro Technologies, Inc., USA). After thawing, viable hepatocytes were transferred to collagen-coated 48-well plates for attachment in plating medium (DMEM stock (Dulbecco's modified Eagle's medium, supplemented with bovine serum albumin, fructose, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonate) (HEPES), and sodium bicarbonate), supplemented with antibiotics, bovine serum, hydrocortisone, insulin and minimum essential medium (MEM) nonessential amino acids). After attachment to the collagen matrix, plating medium was replaced with sandwich medium (plating medium supplemented with VITROGEN) and incubated until use. All incubations were conducted at 37±1° C., 95% air/5% $CO_2$ and saturating humidity.

After establishment of the hepatocyte culture, sandwich medium was removed and the hepatocytes were incubated with incubation solution (DMEM stock supplemented with antibiotics, hydrocortisone, insulin, and MEM non-essential amino acids) containing 0.4, 4.0, or 40 μM metaxalone for 24±1.5 hrs. Incubation solution was aspirated and replaced with incubation solution containing the same concentration of metaxalone and incubated for an additional 24±1.5 hrs. After the metaxalone treatment period, the incubation solution was replaced with 150 μL Krebs-Henseleit (KHB) buffer supplemented with antibiotics, calcium chloride, heptanoic acid, HEPES, and sodium bicarbonate (supplemented KHB) and incubated for 10 minutes. The supplemented KHB was replaced with 150 μL supplemented KHB containing the appropriate isoform-selective substrate and incubated for 4 hrs prior to termination by adding 150 μL ice-cold methanol, except for the CYP2C8 incubations which were terminated by adding 150 μL acetonitrile. Samples were transferred to cryovials and analyzed after storage at −70° C. Three induction replicates were performed at each metaxalone concentration for each cytochrome p450 isozyme.

A table of the substrate, substrate concentration, metabolite formed, and metabolite assay method for each CYP isozyme studied is provided below. All substrates were dissolved in acetonitrile.

| CYP isoform | Isoform-selective substrate | Substrate concentration | Metabolite formed | Metabolite Assay |
|---|---|---|---|---|
| CYP1A2 | Phenacetin | 100 μM | acetaminophen | LC/MS |
| CYP2A6 | Coumarin | 100 μM | 7-hydroxycoumarin, 7-hydroxycoumaringlucuronide, 7-hydroxycoumarin sulfate | HPLC-UV |
| CYP2B6 | S-Mephenytoin | 1 mM | nirvanol | LC/MS |
| CYP2C9 | Tolbutamide | 50 μM | 4'-methylhydroxytolbutamide | LC/MS |
| CYP2C19 | S-Mephenytoin | 100 μM | 4'-hydroxy mephenytoin | LC/MS |
| CYP2D6 | Dextromethorphan | 16 μM | dextrorphan | LC/MS |
| CYP2E1 | Chlorzoxazone | 300 μM | 6-hydroxychlorzoxazone | LC/MS |
| CYP3A4 | Testosterone | 125 μM | 6β-hydroxy testosterone | HPLC-UV |

Metaxalone 100× stock solutions were prepared in methanol as described above and diluted with incubation medium to produce incubation solutions with 0.4, 4.0, and 40 μM metaxalone.

Replicate trials and controls were performed. Positive controls (n=4) were performed to verify that the test system was sensitive to known inducers by testing induction of CYP1A2 and CYP3A4 using 50 μM omeprazole and 25 μM rifampicin, respectively, as inducers with the appropriate isoform-selective substrate. Both positive control test systems showed ≧200% induction. Additionally, reference control samples were included to evaluate inducibility of CYP2B6, CYP2C9, and CYP2C19 in the test system. The reference controls included 1 mM Phenobarbital (CYP2B6) or 25 μM rifampicin as the reference inducer. The reference controls showed a statistically significant amount of induction for each hepatocyte donor for CYP2B6 and CYP2C9, although the amount of induction varied between the three hepatocyte donors for each isozyme. For CYP2C19, rifampin induced CYP2C19 activity in donor 1 and donor 3, but did not induce CYP2C19 activity in donor 2 at a statistically significant level ($p<0.05$ using an unpaired two-tailed t-test).

Results for each cytochrome p450 isozyme are shown in Tables 18–25. Significant induction was observed at these experimental conditions in all three donors for CYP1A2 and in one donor for CYP3A4 at the highest tested concentration. Additionally, significant inhibition in enzyme activity was observed in all three donors for CYP2C9 and in two donors for CYP2D6. Under these experimental conditions, no significant effects on activity of CYP2A6, CYP2B6, CYP2C19, or CYP2E1 were observed after exposure to any of the tested concentrations of metaxalone. Significance of a change in specific activity from that measured for the vehicle control (0 μM metaxalone) was determined using a two-tailed t-test. Mean specific activity values with associated p-values ≦0.05 were deemed to be statistically significant.

TABLE 18

CYP1A2 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Acetaminophen formation: Raw (μM) | Acetaminophen formation: Adjusted (μM) Individual | Acetaminophen formation: Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 (VC) | 0.05388 | 0.0539 | 0.0487 ± 0.00543 | 0.481 | 0.435 ± 0.0485 | 100 |
| | 0.05227 | 0.0523 | | 0.467 | | |
| | 0.04658 | 0.0466 | | 0.416 | | |
| | 0.04203 | 0.0420 | | 0.375 | | |
| 0.4 | 0.05121 | 0.0512 | 0.0537 ± 0.00309 | 0.457 | 0.479 ± 0.0276 | 110 |
| | 0.05264 | 0.0526 | | 0.470 | | |
| | 0.05714 | 0.0571 | | 0.510 | | |
| 4 | 0.07410 | 0.0741 | 0.0638 ± 0.0193 | 0.662 | 0.570 ± 0.172 | 131 |
| | 0.07581 | 0.0758 | | 0.677 | | |
| | 0.04160 | 0.0416 | | 0.371 | | |
| 40 | 0.15156 | 0.152 | 0.161 ± 0.0133 | 1.35 | 1.44 ± 0.119 | 332 |
| | 0.15617 | 0.156 | | 1.39 | | |
| | 0.17659 | 0.177 | | 1.58 | | |
| | | | Donor 2 | | | |
| 0 (VC) | 0.03023 | 0.0302 | 0.0300 ± 0.00305 | 0.270 | 0.267 ± 0.0272 | 100 |
| | 0.03210 | 0.0321 | | 0.287 | | |
| | 0.03193 | 0.0319 | | 0.285 | | |
| | 0.02556 | 0.0256 | | 0.228 | | |
| 0.4 | 0.03165 | 0.0317 | 0.0323 ± 0.000850 | 0.283 | 0.289 ± 0.00759 | 108 |
| | 0.03208 | 0.0321 | | 0.286 | | |
| | 0.03329 | 0.0333 | | 0.297 | | |
| 4 | 0.03346 | 0.0335 | 0.0340 ± 0.00198 | 0.299 | 0.304 ± 0.0177 | 113 |
| | 0.03619 | 0.0362 | | 0.323 | | |
| | 0.03234 | 0.0323 | | 0.289 | | |

TABLE 18-continued

CYP1A2 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Acetaminophen formation Raw (μM) | Adjusted (μM) Individual | Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 40 | 0.06015 | 0.0602 | 0.0589 ± 0.00795 | 0.537 | 0.526 ± 0.0710 | 197 |
| | 0.06616 | 0.0662 | | 0.591 | | |
| | 0.05040 | 0.0504 | | 0.450 | | |
| | | | Donor 3 | | | |
| 0 | 0.04357 | 0.0436 | 0.0410 ± 0.00447 | 0.389 | 0.366 ± 0.0399 | 100 |
| (VC) | 0.04576 | 0.0458 | | 0.409 | | |
| | 0.03607 | 0.0361 | | 0.322 | | |
| | 0.03849 | 0.0385 | | 0.344 | | |
| 0.4 | 0.04030 | 0.0403 | 0.0438 ± 0.00361 | 0.360 | 0.391 ± 0.0322 | 107 |
| | 0.04347 | 0.0435 | | 0.388 | | |
| | 0.04750 | 0.0475 | | 0.424 | | |
| 4 | 0.04411 | 0.0441 | 0.0443 ± 0.000214 | 0.394 | 0.396 ± 0.00191 | 108 |
| | 0.04453 | 0.0445 | | 0.398 | | |
| | 0.04425 | 0.0443 | | 0.395 | | |
| 40 | 0.12276 | 0.123 | 0.122 ± 0.00365 | 1.10 | 1.09 ± 0.0326 | 297 |
| | 0.11776 | 0.118 | | 1.05 | | |
| | 0.12487 | 0.125 | | 1.11 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 19

CYP2A6 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Total Metabolite formation Raw (μM) | Adjusted (μM) Individual | Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Total Metabolite Formation: Donor 1 | | | |
| 0 | 0.0171[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| (VC) | 0.000[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |
| 0.4 | 0.000[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.000[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |
| 4 | 0.000[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.000[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |
| 40 | 0.000[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.000[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |
| | | | Total Metabolite Formation: Donor 2 | | | |
| 0 | 0.0381[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| (VC) | 0.0413[d] | <0.300 | | <2.68 | | |
| | 0.0365[d] | <0.300 | | <2.68 | | |
| | 0.0320[d] | <0.300 | | <2.68 | | |
| 0.4 | 0.0225[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.0381[d] | <0.300 | | <2.68 | | |
| | 0.0381[d] | <0.300 | | <2.68 | | |
| 4 | 0.0344[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.0353[d] | <0.300 | | <2.68 | | |
| | 0.0297[d] | <0.300 | | <2.68 | | |
| 40 | 0.0293[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.0266[d] | <0.300 | | <2.68 | | |
| | 0.0333[d] | <0.300 | | <2.68 | | |
| | | | Total Metabolite Formation: Donor 3 | | | |
| 0 | 0.000[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| (VC) | 0.0196[d] | <0.300 | | <2.68 | | |
| | 0.0237[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |

TABLE 19-continued

CYP2A6 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Total Metabolite formation: Raw (μM) | Adjusted (μM): Individual | Adjusted (μM): Mean ± SD | Specific Activity (pmol/min/mg protein): Individual | Specific Activity (pmol/min/mg protein): Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 0.4 | 0.0216[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.0182[d] | <0.300 | | <2.68 | | |
| | 0.0182[d] | <0.300 | | <2.68 | | |
| 4 | 0.000[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.0197[d] | <0.300 | | <2.68 | | |
| | 0.0162[d] | <0.300 | | <2.68 | | |
| 40 | 0.000[d] | <0.300 | <0.300 ± 0.000 | <2.68 | <2.68 ± 0.000 | 100 |
| | 0.0188[d] | <0.300 | | <2.68 | | |
| | 0.000[d] | <0.300 | | <2.68 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)

[d]The observed analyzed value (μM) for all metabolites were below the lowest concentration on the corresponding standard curve.

Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 20

CYP2B6 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Nirvanol formation: Raw (μM) | Adjusted (μM): Individual | Adjusted (μM): Mean ± SD | Specific Activity (pmol/min/mg protein): Individual | Specific Activity (pmol/min/mg protein): Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 (VC) | 0.03230 | 0.0323 | 0.0319 ± 0.00156 | 0.288 | 0.285 ± 0.0139 | 100 |
| | 0.03384 | 0.0338 | | 0.302 | | |
| | 0.03014 | 0.0301 | | 0.269 | | |
| | 0.03141 | 0.0314 | | 0.280 | | |
| 0.4 | 0.03380 | 0.0338 | 0.0340 ± 0.000883 | 0.302 | 0.304 ± 0.00789 | 107 |
| | 0.03329 | 0.0333 | | 0.297 | | |
| | 0.03501 | 0.0350 | | 0.313 | | |
| 4 | 0.02742 | 0.0274 | 0.0305 ± 0.00272 | 0.245 | 0.273 ± 0.0243 | 95.7 |
| | 0.03241 | 0.0324 | | 0.289 | | |
| | 0.03178 | 0.0318 | | 0.284 | | |
| 40 | 0.03233 | 0.0323 | 0.0310 ± 0.00204 | 0.289 | 0.277 ± 0.0182 | 97.1 |
| | 0.03203 | 0.0320 | | 0.286 | | |
| | 0.02866 | 0.0287 | | 0.256 | | |
| | | | Donor 2 | | | |
| 0 (VC) | 0.02927 | 0.0293 | 0.0289 ± 0.00230 | 0.261 | 0.258 ± 0.0205 | 100 |
| | 0.02920 | 0.0292 | | 0.261 | | |
| | 0.03137 | 0.0314 | | 0.280 | | |
| | 0.02582 | 0.0258 | | 0.231 | | |
| 0.4 | 0.02544 | 0.0254 | 0.0306 ± 0.00559 | 0.227 | 0.273 ± 0.0499 | 106 |
| | 0.02986 | 0.0299 | | 0.267 | | |
| | 0.03654 | 0.0365 | | 0.326 | | |
| 4 | 0.02852 | 0.0285 | 0.0281 ± 0.000884 | 0.255 | 0.250 ± 0.00790 | 97.0 |
| | 0.02703 | 0.0270 | | 0.241 | | |
| | 0.02860 | 0.0286 | | 0.255 | | |
| 40 | 0.00341[a] | <0.0250 | <0.0250 ± 0.000 | <0.223 | <0.223 ± 0.000 | <86.5 |
| | 0.00320[a] | <0.0250 | | <0.223 | | |
| | 0.00330[a] | <0.0250 | | <0.223 | | |
| | | | Donor 3 | | | |
| 0 (VC) | 0.02349[a] | <0.0250 | <0.0252 ± 0.000435 | <0.223 | <0.225 ± 0.00388 | 100 |
| | 0.02587 | 0.0259 | | 0.231 | | |
| | 0.02376[a] | <0.0250 | | <0.223 | | |
| | 0.02236[a] | <0.0250 | | <0.223 | | |
| 0.4 | 0.02177[a] | <0.0250 | <0.0250 ± 0.000 | <0.223 | <0.223 ± 0.000 | 99.1 |
| | 0.02343[a] | <0.0250 | | <0.223 | | |
| | 0.02326[a] | <0.0250 | | <0.223 | | |
| 4 | 0.02392[a] | <0.0250 | <0.0250 ± 0.000 | <0.223 | <0.223 ± 0.000 | 99.1 |
| | 0.02490[a] | <0.0250 | | <0.223 | | |
| | 0.02229[a] | <0.0250 | | <0.223 | | |

TABLE 20-continued

CYP2B6 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Nirvanol formation Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 40 | 0.02005[a] | <0.0250 | <0.0250 ± 0.000 | <0.223 | <0.223 ± 0.000 | 99.1 |
| | 0.01976[a] | <0.0250 | | <0.223 | | |
| | 0.02169[a] | <0.0250 | | <0.223 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
[a]The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.025 μM).
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 21

CYP2C9 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | 4'-Methylhydroxytolbutamide formation Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 | 0.01215 | 0.0122 | 0.0137 ± 0.00161 | 0.108 | 0.122 ± 0.0144 | 100 |
| (VC) | 0.01502 | 0.0150 | | 0.134 | | |
| | 0.01513 | 0.0151 | | 0.135 | | |
| | 0.01245 | 0.0125 | | 0.111 | | |
| 0.4 | 0.01557 | 0.0156 | 0.0147 ± 0.000753 | 0.139 | 0.132 ± 0.00672 | 108 |
| | 0.01410 | 0.0141 | | 0.126 | | |
| | 0.01455 | 0.0146 | | 0.130 | | |
| 4 | 0.01331 | 0.0133 | 0.0137 ± 0.00136 | 0.119 | 0.122 ± 0.0121 | 100 |
| | 0.01523 | 0.0152 | | 0.136 | | |
| | 0.01261 | 0.0126 | | 0.113 | | |
| 40 | 0.00931[a] | <0.0100 | <0.0100 ± 0.0000346 | <0.0893 | <0.0895 ± 0.000309 | <73.2 |
| | 0.00952[a] | <0.0100 | | <0.0893 | | |
| | 0.01006 | 0.0101 | | 0.0898 | | |
| | | | Donor 2 | | | |
| 0 | 0.05192 | 0.0519 | 0.0491 ± 0.00479 | 0.464 | 0.438 ± 0.0428 | 100 |
| (VC) | 0.04864 | 0.0486 | | 0.434 | | |
| | 0.05325 | 0.0533 | | 0.475 | | |
| | 0.04250 | 0.0425 | | 0.379 | | |
| 0.4 | 0.04819 | 0.0482 | 0.0474 ± 0.00223 | 0.430 | 0.423 ± 0.0200 | 96.6 |
| | 0.04489 | 0.0449 | | 0.401 | | |
| | 0.04915 | 0.0492 | | 0.439 | | |
| 4 | 0.04634 | 0.0463 | 0.0456 ± 0.000864 | 0.414 | 0.407 ± 0.00772 | 92.9 |
| | 0.04581 | 0.0458 | | 0.409 | | |
| | 0.04465 | 0.0447 | | 0.399 | | |
| 40 | 0.02917 | 0.0292 | 0.0296 ± 0.000651 | 0.260 | 0.265 ± 0.00581 | 60.4 |
| | 0.02936 | 0.0294 | | 0.262 | | |
| | 0.03038 | 0.0304 | | 0.271 | | |
| | | | Donor 3 | | | |
| 0 | 0.02021 | 0.0202 | 0.0181 ± 0.00206 | 0.180 | 0.162 ± 0.0184 | 100 |
| (VC) | 0.01700 | 0.0170 | | 0.152 | | |
| | 0.01952 | 0.0195 | | 0.174 | | |
| | 0.01586 | 0.0159 | | 0.142 | | |
| 0.4 | 0.02067 | 0.0207 | 0.0201 ± 0.00125 | 0.185 | 0.179 ± 0.0111 | 111 |
| | 0.02096 | 0.0210 | | 0.187 | | |
| | 0.01867 | 0.0187 | | 0.167 | | |
| 4 | 0.01807 | 0.0181 | 0.0187 ± 0.00235 | 0.161 | 0.167 ± 0.0210 | 103 |
| | 0.02129 | 0.0213 | | 0.190 | | |
| | 0.01671 | 0.0167 | | 0.149 | | |
| 40 | 0.01364 | 0.0136 | 0.0142 ± 0.000560 | 0.122 | 0.127 ± 0.00500 | 78.4 |
| | 0.01432 | 0.0143 | | 0.128 | | |
| | 0.01475 | 0.0148 | | 0.132 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
[a]The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.01 μM).
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 22

CYP2C19 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | 4'-Hydroxymephenytoin formation Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 (VC) | 0.00025* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00058* | <0.0500 | | <0.446 | | |
| | 0.00114* | <0.0500 | | <0.446 | | |
| | 0.00058* | <0.0500 | | <0.446 | | |
| 0.4 | 0.00708* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.01319* | <0.0500 | | <0.446 | | |
| | 0.01861* | <0.0500 | | <0.446 | | |
| 4 | 0.01649* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00029* | <0.0500 | | <0.446 | | |
| | 0.00064* | <0.0500 | | <0.446 | | |
| 40 | 0.00057* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00031* | <0.0500 | | <0.446 | | |
| | 0.00037* | <0.0500 | | <0.446 | | |
| | | | Donor 2 | | | |
| 0 (VC) | N/A* | N/A | <0.0500 ± 0.000 | N/A | <0.446 ± 0.000 | 100 |
| | 0.01146* | <0.0500 | | <0.446 | | |
| | 0.01456* | <0.0500 | | <0.446 | | |
| | N/A* | N/A | | N/A | | |
| 0.4 | 0.00765* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00779* | <0.0500 | | <0.446 | | |
| | 0.00808* | <0.0500 | | <0.446 | | |
| 4 | 0.00775* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00744* | <0.0500 | | <0.446 | | |
| | 0.00773* | <0.0500 | | <0.446 | | |
| 40 | 0.00697* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00840* | <0.0500 | | <0.446 | | |
| | 0.00790* | <0.0500 | | <0.446 | | |
| | | | Donor 3 | | | |
| 0 (VC) | 0.00026* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00000* | <0.0500 | | <0.446 | | |
| | 0.00000* | <0.0500 | | <0.446 | | |
| | 0.00000* | <0.0500 | | <0.446 | | |
| 0.4 | 0.00000* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00000* | <0.0500 | | <0.446 | | |
| | 0.00023* | <0.0500 | | <0.446 | | |
| 4 | 0.00000* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00000* | <0.0500 | | <0.446 | | |
| | 0.00000* | <0.0500 | | <0.446 | | |
| 40 | 0.00191* | <0.0500 | <0.0500 ± 0.000 | <0.446 | <0.446 ± 0.000 | 100 |
| | 0.00000* | <0.0500 | | <0.446 | | |
| | 0.00000* | <0.0500 | | <0.446 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
*The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.05 μM).
*Sample lost after preparation.
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 23

CYP2D6 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Dextrorphan formation Raw (μM) | Adjusted (μM) Individual | Adjusted (μM) Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Specific Activity (pmol/min/mg protein) Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 (VC) | 0.00772* | <0.0100 | <0.0100 ± 0.000 | <0.0893 | <0.0893 ± 0.000 | 100 |
| | 0.00796* | <0.0100 | | <0.0893 | | |
| | 0.00736* | <0.0100 | | <0.0893 | | |
| | 0.00724* | <0.0100 | | <0.0893 | | |

TABLE 23-continued

CYP2D6 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | Dextrorphan formation Raw (μM) | Adjusted (μM) Individual | Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 0.4 | 0.00809[a] | <0.0100 | <0.0100 ± 0.000 | <0.0893 | <0.0893 ± 0.000 | 100 |
| | 0.00749[a] | <0.0100 | | <0.0893 | | |
| | 0.00853[a] | <0.0100 | | <0.0893 | | |
| 4 | 0.00832[a] | <0.0100 | <0.0100 ± 0.000 | <0.0893 | <0.0893 ± 0.000 | 100 |
| | 0.00721[a] | <0.0100 | | <0.0893 | | |
| | 0.00744[a] | <0.0100 | | <0.0893 | | |
| 40 | 0.00398[a] | <0.0100 | <0.0100 ± 0.000 | <0.0893 | <0.0893 ± 0.000 | 100 |
| | 0.00205[a] | <0.0100 | | <0.0893 | | |
| | 0.00520[a] | <0.0100 | | <0.0893 | | |
| | | | Donor 2 | | | |
| 0 | 0.01286 | 0.0129 | 0.0139 ± 0.00152 | 0.115 | 0.124 ± 0.0136 | 100 |
| (VC) | 0.01432 | 0.0143 | | 0.128 | | |
| | 0.01581 | 0.0158 | | 0.141 | | |
| | 0.01247 | 0.0125 | | 0.111 | | |
| 0.4 | 0.01302 | 0.0130 | 0.0133 ± 0.000485 | 0.116 | 0.119 ± 0.00433 | 95.9 |
| | 0.01302 | 0.0130 | | 0.116 | | |
| | 0.01386 | 0.0139 | | 0.124 | | |
| 4 | 0.01361 | 0.0136 | 0.0143 ± 0.000589 | 0.122 | 0.128 ± 0.00526 | 103 |
| | 0.01468 | 0.0147 | | 0.131 | | |
| | 0.01457 | 0.0146 | | 0.130 | | |
| 40 | 0.00998[a] | <0.0100 | <0.0102 ± 0.000260 | <0.0893 | <0.0906 ± 0.00232 | <73.2 |
| | 0.00956[a] | <0.0100 | | <0.0893 | | |
| | 0.01045 | 0.0105 | | 0.0933 | | |
| | | | Donor 3 | | | |
| 0 | 0.07011 | 0.0701 | 0.0665 ± 0.00607 | 0.626 | 0.594 ± 0.0542 | 100 |
| (VC) | 0.05856 | 0.0586 | | 0.523 | | |
| | 0.07219 | 0.0722 | | 0.645 | | |
| | 0.06505 | 0.0651 | | 0.581 | | |
| 0.4 | 0.06218 | 0.0622 | 0.0657 ± 0.00305 | 0.555 | 0.586 ± 0.0272 | 98.8 |
| | 0.06688 | 0.0669 | | 0.597 | | |
| | 0.06789 | 0.0679 | | 0.606 | | |
| 4 | 0.06071 | 0.0607 | 0.0597 ± 0.00164 | 0.542 | 0.533 ± 0.0146 | 89.8 |
| | 0.06060 | 0.0606 | | 0.541 | | |
| | 0.05782 | 0.0578 | | 0.516 | | |
| 40 | 0.05087 | 0.0509 | 0.0489 ± 0.00347 | 0.454 | 0.436 ± 0.0310 | 73.5 |
| | 0.05088 | 0.0509 | | 0.454 | | |
| | 0.04486 | 0.0449 | | 0.401 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
[a]The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.01 μM).
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 24

CYP2E1 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | 6-Hydroxychlorzoxazone formation Raw (μM) | Adjusted (μM) Individual | Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 | 0.28067 | 0.281 | 0.283 ± 0.00460 | 2.51 | 2.53 ± 0.0411 | 100 |
| (VC) | 0.28793 | 0.288 | | 2.57 | | |
| | 0.28627 | 0.286 | | 2.56 | | |
| | 0.27817 | 0.278 | | 2.48 | | |
| 0.4 | 0.28854 | 0.289 | 0.277 ± 0.0279 | 2.58 | 2.47 ± 0.249 | 97.8 |
| | 0.29749 | 0.297 | | 2.66 | | |
| | 0.24529 | 0.245 | | 2.19 | | |
| 4 | 0.28784 | 0.288 | 0.295 ± 0.0236 | 2.57 | 2.64 ± 0.210 | 104 |
| | 0.27623 | 0.276 | | 2.47 | | |
| | 0.32160 | 0.322 | | 2.87 | | |
| 40 | 0.28453 | 0.285 | 0.294 ± 0.00876 | 2.54 | 2.63 ± 0.0782 | 104 |
| | 0.29753 | 0.298 | | 2.66 | | |
| | 0.30121 | 0.301 | | 2.69 | | |

TABLE 24-continued

CYP2E1 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | 6-Hydroxychlorzoxazone formation: Raw (μM) | Adjusted (μM): Individual | Adjusted (μM): Mean ± SD | Specific Activity (pmol/min/mg protein): Individual | Specific Activity (pmol/min/mg protein): Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 2 | | | |
| 0 | 0.07385 | 0.0739 | 0.0748 ± 0.00211 | 0.659 | 0.668 ± 0.0188 | 100 |
| (VC) | 0.07610 | 0.0761 | | 0.679 | | |
| | 0.07690 | 0.0769 | | 0.687 | | |
| | 0.07229 | 0.0723 | | 0.645 | | |
| 0.4 | 0.07071 | 0.0707 | 0.0776 ± 0.00753 | 0.631 | 0.693 ± 0.0673 | 104 |
| | 0.07649 | 0.0765 | | 0.683 | | |
| | 0.08565 | 0.0857 | | 0.765 | | |
| 4 | 0.06315 | 0.0632 | 0.0670 ± 0.00355 | 0.564 | 0.598 ± 0.0317 | 89.6 |
| | 0.06775 | 0.0678 | | 0.605 | | |
| | 0.07013 | 0.0701 | | 0.626 | | |
| 40 | 0.06247 | 0.0625 | 0.0745 ± 0.0141 | 0.558 | 0.665 ± 0.126 | 99.6 |
| | 0.07091 | 0.0709 | | 0.633 | | |
| | 0.09003 | 0.0900 | | 0.804 | | |
| | | | Donor 3 | | | |
| 0 | 0.05899 | 0.0590 | 0.0570 ± 0.00420 | 0.527 | 0.509 ± 0.0375 | 100 |
| (VC) | 0.06077 | 0.0608 | | 0.543 | | |
| | 0.05718 | 0.0572 | | 0.511 | | |
| | 0.05110 | 0.0511 | | 0.456 | | |
| 0.4 | 0.05031 | 0.0503 | 0.0517 ± 0.00140 | 0.449 | 0.462 ± 0.0125 | 90.7 |
| | 0.05310 | 0.0531 | | 0.474 | | |
| | 0.05169 | 0.0517 | | 0.462 | | |
| 4 | 0.05245 | 0.0525 | 0.0500 ± 0.00389 | 0.468 | 0.446 ± 0.0348 | 87.7 |
| | 0.05202 | 0.0520 | | 0.464 | | |
| | 0.04550 | 0.0455 | | 0.406 | | |
| 40 | 0.05260 | 0.0526 | 0.0535 ± 0.00164 | 0.470 | 0.478 ± 0.0146 | 93.9 |
| | 0.05541 | 0.0554 | | 0.495 | | |
| | 0.05254 | 0.0525 | | 0.469 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol)
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

TABLE 25

CYP3A4 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | 6β-Hydroxytestosterone formation: Raw (μM) | Adjusted (μM): Individual | Adjusted (μM): Mean ± SD | Specific Activity (pmol/min/mg protein): Individual | Specific Activity (pmol/min/mg protein): Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| | | | Donor 1 | | | |
| 0 | 0.05693[a] | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| (VC) | 0.05726[a] | <0.100 | | <0.893 | | |
| | 0.05367[a] | <0.100 | | <0.893 | | |
| | 0.04590[a] | <0.100 | | <0.893 | | |
| 0.4 | 0.05415[a] | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| | 0.06053[a] | <0.100 | | <0.893 | | |
| | 0.05911[a] | <0.100 | | <0.893 | | |
| 4 | 0.05783[a] | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| | 0.05948[a] | <0.100 | | <0.893 | | |
| | 0.05705[a] | <0.100 | | <0.893 | | |
| 40 | 0.06888[a] | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| | 0.06424[a] | <0.100 | | <0.893 | | |
| | 0.06511[a] | <0.100 | | <0.893 | | |
| | | | Donor 2 | | | |
| 0 | 0.12401 | 0.124 | <0.117 ± 0.0141 | 1.11 | <1.05 ± 0.126 | 100 |
| (VC) | 0.13222 | 0.132 | | 1.18 | | |
| | 0.07973[a] | <0.100 | | <0.893 | | |
| | 0.11219 | 0.112 | | 1.00 | | |
| 0.4 | 0.12083 | 0.121 | 0.134 ± 0.0122 | 1.08 | 1.20 ± 0.109 | >115 |
| | 0.14424 | 0.144 | | 1.29 | | |
| | 0.13828 | 0.138 | | 1.23 | | |

TABLE 25-continued

CYP3A4 Activity in Cryopreserved Human Hepatocytes

| Metaxalone (μM) | 6β-Hydroxytestosterone formation Raw (μM) | Adjusted (μM) Individual | Mean ± SD | Specific Activity (pmol/min/mg protein) Individual | Mean ± SD | Percent of VC |
|---|---|---|---|---|---|---|
| 4 | 0.10953 | 0.110 | 0.116 ± 0.00524 | 0.978 | 1.03 ± 0.0468 | >98.7 |
| | 0.11883 | 0.119 | | 1.06 | | |
| | 0.11837 | 0.118 | | 1.06 | | |
| 40 | 0.14198 | 0.142 | 0.141 ± 0.00273 | 1.27 | 1.26 ± 0.0244 | >121 |
| | 0.14356 | 0.144 | | 1.28 | | |
| | 0.13824 | 0.138 | | 1.23 | | |
| | | | Donor 3 | | | |
| 0 | 0.06064* | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| (VC) | 0.05981* | <0.100 | | <0.893 | | |
| | 0.06402* | <0.100 | | <0.893 | | |
| | 0.08660* | <0.100 | | <0.893 | | |
| 0.4 | 0.05106* | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| | 0.08255* | <0.100 | | <0.893 | | |
| | 0.05998* | <0.100 | | <0.893 | | |
| 4 | 0.06298* | <0.100 | <0.100 ± 0.000 | <0.893 | <0.893 ± 0.000 | 100 |
| | 0.05381* | <0.100 | | <0.893 | | |
| | 0.07264* | <0.100 | | <0.893 | | |
| 40 | 0.05587* | <0.100 | <0.101 ± 0.00238 | <0.893 | <0.905 ± 0.0213 | 101 |
| | 0.10413 | 0.104 | | 0.930 | | |
| | 0.08088* | <0.100 | | <0.893 | | |

Abbreviations: SD, standard deviation; VC, vehicle control (1% Methanol);
*The observed analyzed value (μM) was below the lowest concentration on the standard curve (0.1 μM).
Note: For all calculations above, the resulting values are shown with at least three significant figures for display purposes only.

Table 18 presents the results for CYP1A2. Under these experimental conditions, exposure to metaxalone at 40 μM induced CYP1A2 activity in human hepatocytes prepared from Donors 1, 2, and 3. For each of the three donors, the increases in CYP1A2 activity by metaxalone at 0.4 and 4 μM were not statistically significant (p>0.05; unpaired two-tailed t test).

Table 25 presents the results for CYP3A4. Metaxalone at the concentration of 40 μM induced CYP3A4 activity by about 21% in one of three donors tested, Donor 2. Therefore under these experimental conditions, exposure to metaxalone at 40 μM induced CYP3A4 activity in human hepatocytes prepared from Donor 2. The increase in CYP3A4 activity following treatment with metaxalone at 0.4 μM for Donor 2 was not statistically significant (p>0.05; unpaired two-tailed t test). CYP3A4 activity in the vehicle controls for Donor 1 and Donor 3 were below the lower limit of quantitation. Exposure of hepatocytes from Donors 1 and 3 to metaxalone at the concentrations tested did not induce CYP3A4 activity since the activity following treatment with metaxalone was still below the lower limit of quantitation at each tested concentration.

Table 21 presents the results for CYP2C9. Under these experimental conditions, exposure to metaxalone at 40 μM significantly reduced CYP2C9 activity in human hepatocytes prepared from Donors 1, 2, and 3. The observed changes in CYP2C9 activity following exposure to metaxalone at 0.4 and 4 μM were not statistically significant (p>0.05; two-tailed t test). Thus, under these experimental conditions, exposure to metaxalone at 40 μM inhibited CYP2C9 activity.

Table 23 presents the results for CYP2D6. CYP2D6 activity was below the lower limit of quantitation in the vehicle controls and for the metaxalone-exposed samples for Donor 1. However, under these experimental conditions, exposure to metaxalone at 40 μM significantly reduced CYP2D6 activity in human hepatocytes prepared from Donors 2 and 3. The observed changes in CYP2D6 activity following exposure to metaxalone at 0.4 and 4 μM were not statistically significant (p>0.05; two-tailed t test). Thus, under these experimental conditions, exposure to metaxalone at 40 μM inhibited CYP2D6 activity.

Any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

We claim:

1. A method of using metaxalone for treating a patient's condition, comprising

providing a patient with metaxalone; and

informing the patient or a medical care worker that metaxalone affects activity of a cytochrome p450 isozyme, and that administration of metaxalone with a substance that affects activity of a cytochrome p450 isozyme can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both.

2. The method of claim 1, wherein the substance is an active agent with a narrow therapeutic index.

3. The method of claim 2, wherein the substance is a substrate of CYP1A2, CYP3A4, CYP2B6, CYP2C19, CYP2D6, CYP2E1, or CYP2C9.

4. The method of claim 2, wherein the substance is warfarin, phenytoin, fosphenytoin, thioridazine, or theophylline.

5. A method of using metaxalone to treat a patient's condition, comprising:

providing a patient with metaxalone; and

informing the patient or a medical care worker that a cytochrome p450 isozyme metabolizing metaxalone is CYP1A2 or CYP2C19 and that administration of metaxalone and a substance that is a substrate, inhibitor, or inducer of CYP1A2 or CYP2C19 can affect plasma concentration, safety, efficacy or any combination thereof of metaxalone, the substance, or both.

**6**. A method of using metaxalone to treat a patient's condition, comprising:

providing a patient with metaxalone; and

informing the patient or a medical care worker that metaxalone is an inhibitor, inducer, or substrate of a cytochrome p450 isozyme and administration of metaxalone with a substance that is an inhibitor, inducer, or substrate of the cytochrome p450 isozyme can affect the plasma concentration, safety or efficacy of the substance.

**7**. The method of claim **6**, wherein the cytochrome p450 isozyme is CYP1A2, CYP3A4, CYP2B6, CYP2C19, CYP2D6, CYP2E1, or CYP2C9.

**8**. The method of claim **6**, wherein the substance is an active agent with a narrow therapeutic index.

**9**. The method of claim **8**, wherein the substance is a substrate of CYP1A2, CYP3A4, CYP2B6, CYP2C19, CYP2D6, CYP2E1, or CYP2C9.

**10**. The method of claim **8**, wherein the active agent with the narrow therapeutic index is an inhibitor of the cytochrome p450 isozyme.

**11**. The method of claim **8**, wherein the active agent with the narrow therapeutic index is an inducer of the cytochrome p450 isozyme.

**12**. The method of claim **8**, wherein the active agent with the narrow therapeutic index is a substrate of the cytochrome p450 isozyme.

**13**. The method of claim **8**, wherein the substance is warfarin, phenytoin, fosphenytoin, thioridazine, or theophylline.

**14**. The method of claim **1**, wherein metaxalone is an inducer of the cytochrome p450 isozyme.

**15**. The method of claim **14**, wherein the cytochrome p450 isozyme is CYP1A2 or CYP3A4.

**16**. The method of claim **1**, wherein metaxalone is an inhibitor of the cytochrome p450 isozyme.

**17**. The method of claim **16**, wherein the cytochrome p450 isozyme is CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP2C9, CYP2E1, or CYP3A4.

**18**. The method of claim **1**, wherein metaxalone is a substrate of the cytochrome p450 isozyme.

**19**. The method of claim **18**, wherein the cytochrome p450 isozyme is CYP1A2 or CYP2C19.

**20**. The method of claim **1**, wherein the patient is a human patient.

**21**. The method of claim **1**, wherein the patient is a patient with a musculoskeletal condition.

**22**. The method of claim **1**, wherein the patient is a patient receiving metaxalone therapy.

* * * * *

# EXHIBIT C

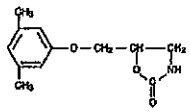

## Metaxalone Tablets

℞ only

**DESCRIPTION**

Metaxalone tablets are available as an 800 mg tablet.

Chemically, metaxalone is 5-[(3,5-dimethylphenoxy)methyl]-2-oxazolidinone. The empirical formula is $C_{12}H_{15}NO_3$, which corresponds to a molecular weight of 221.25. The structural formula is:



Metaxalone is a white to almost white, odorless crystalline powder freely soluble in chloroform, soluble in methanol and in 95% ethanol, but practically insoluble in ether or water.

Each tablet contains 800 mg metaxalone and the following inactive ingredients: corn starch, alginic acid, acacia, sodium starch glycolate, magnesium stearate and FD&C red No. 40 aluminum lake.

**CLINICAL PHARMACOLOGY**

**Mechanism of Action**

The mechanism of action of metaxalone in humans has not been established, but may be due to general central nervous system depression. Metaxalone has no direct action on the contractile mechanism of striated muscle, the motor end plate or the nerve fiber.

**Pharmacokinetics**

The pharmacokinetics of metaxalone have been evaluated in healthy adult volunteers after single dose administration of metaxalone tablets under fasted and fed conditions at doses ranging from 400 mg to 800 mg.

***Absorption:*** Peak plasma concentrations of metaxalone occur approximately 3 hours after a 400 mg oral dose under fasted conditions. Thereafter, metaxalone concentrations decline log-linearly with a terminal half-life of 8.0 ± 4.8 hours. Doubling the dose of metaxalone tablets from 400 mg to 800 mg results in a roughly proportional increase in metaxalone exposure as indicated by peak plasma concentrations ($C_{max}$) and area under the curve (AUC). Dose proportionality at doses above 800 mg has not been studied. The absolute bioavailability of metaxalone is not known.

The single-dose pharmacokinetic parameters of metaxalone in two groups of healthy volunteers are shown in Table 1.

**Table 1: Mean (%CV) Metaxalone Pharmacokinetic Parameters**

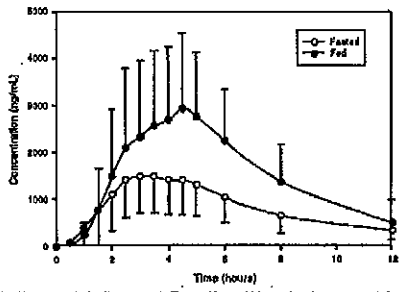
| Dose (mg) | $C_{max}$ (ng/mL) | $T_{max}$ (h) | AUC$_\infty$ (ng·h/mL) | $T_{1/2}$ (h) | CL/F (L/h) |
|---|---|---|---|---|---|
| 400[1] | 963 (53) | 3.3 (35) | 7479 (61) | 9.0 (53) | 68 (50) |
| 800[2] | 1816 (49) | 3.0 (39) | 15044 (46) | 8.0 (58) | 66 (51) |

[1]Subjects received 1x400 mg tablet under fasted conditions (N=42)
[2]Subjects received 2x400 mg tablets under fasted conditions (N=58)

***Food Effects:*** A randomized, two-way, crossover study was conducted in 42 healthy volunteers (31 males, 11 females) administered one 400 mg metaxalone tablet under fasted conditions and following a standard high-fat breakfast. Subjects ranged in age from 18 to 48 years (mean age = 23.5 ± 5.7 years). Compared to fasted conditions, the presence of a high fat meal at the time of drug administration increased $C_{max}$ by 177.5% and increased AUC ($AUC_{0-t}$, $AUC_\infty$) by 123.5% and 115.4%, respectively. Time-to-peak concentration ($T_{max}$) was also delayed (4.3 h *versus* 3.3 h) and terminal half-life was decreased (2.4 h versus 9.0 h) under fed conditions compared to fasted.

In a second food effect study of similar design, two 400 mg metaxalone tablets (800 mg) were administered to healthy volunteers (N=59, 37 males, 22 females), ranging in age from 18 to 60 years (mean age = 25.6 ± 8.7 years). Compared to fasted conditions, the presence of a high fat meal at the time of drug administration increased $C_{max}$ by 193.6% and increased AUC ($AUC_{0-t}$, $AUC_\infty$) by 148.4% and 142.2%, respectively. Time-to-peak concentration ($T_{max}$) was also delayed (4.9 h *versus* 3.0 h) and terminal half-life was decreased (4.2 h *versus* 8.0 h) under fed conditions compared to fasted conditions. Similar food effect results were observed in the above study when one metaxalone 800 mg tablet was administered in place of two metaxalone 400 mg tablets. The increase in metaxalone exposure coinciding with a reduction in half-life may be attributed to more complete absorption of metaxalone in the presence of a high fat meal (Figure 1).

Figure 1. Mean (SD) Concentrations of Metaxalone following an 800 mg Dose under Fasted and Fed Conditions




***Distribution, Metabolism and Excretion:*** Although plasma protein binding and absolute bioavailability of metaxalone are not known, the apparent volume of distribution ($V/F \sim 800$ L) and lipophilicity (log P = 2.42) of metaxalone suggest that the drug is extensively distributed in the tissues. Metaxalone is metabolized by the liver and excreted in the urine as unidentified metabolites. Hepatic Cytochrome P450 enzymes play a role in the metabolism of metaxalone. Specifically, CYP1A2, CYP2D6, CYP2E1, and CYP3A4 and, to a lesser extent, CYP2C8, CYP2C9, and CYP2C19 appear to metabolize metaxalone.

Metaxalone does not significantly inhibit major CYP enzymes such as CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CPY2E1, and CYP3A4. Metaxalone does not significantly induce major CYP enzymes such as CYP1A2, CYP2B6, and CYP3A4 in vitro.

**Pharmacokinetics in Special Populations**

***Age:*** The effects of age on the pharmacokinetics of metaxalone were determined following single administration of two 400 mg tablets (800 mg) under fasted and fed conditions. The results were analyzed separately, as well as in combination with the results from three other studies. Using the combined data, the results indicate that the pharmacokinetics of metaxalone are significantly more affected by age under fasted conditions than under fed conditions, with bioavailability under fasted conditions increasing with age.

The bioavailability of metaxalone under fasted and fed conditions in three groups of healthy volunteers of varying age is shown in Table 2.


**Table 2: Mean (%CV) Pharmacokinetics Parameters Following Single Administration of Two 400 mg Metaxalone Tablets (800 mg) under Fasted and Fed Conditions**

| | Younger Volunteers | | Older Volunteers | | | |
|---|---|---|---|---|---|---|
| Age (years) | 25.6 ± 8.7 | | 39.3 ± 10.8 | | 71.6 ± 5.0 | |
| N | 59 | | 21 | | 23 | |
| Food | Fasted | Fed | Fasted | Fed | Fasted | Fed |
| $C_{max}$ (ng/mL) | 1618 | 3510 | 2719 | 2915 | 3168 | 3680 |
| | (43) | (41) | (46) | (55) | (43) | (59) |
| $T_{MAX}$ (h) | 3.0 | 4.9 | 3.0 | 8.7 | 2.6 | 6.5 |
| | (59) | (46) | (40) | (91) | (30) | (67) |
| $AUC_{0-t}$ (ng.h/mL) | 14531 | 20883 | 19836 | 20482 | 23797 | 24340 |
| | (47) | (41) | (40) | (37) | (45) | (48) |
| $AUC_{\infty}$ (ng.h/mL) | 15045 | 20833 | 20490 | 20815 | 24184 | 24704 |
| | (46) | (41) | (39) | (37) | (44) | (47) |

***Gender:*** The effect of gender on the pharmacokinetics of metaxalone was assessed in an open label study, in which 48 healthy adult volunteers (24 males, 24 females) were administered two metaxalone 400 mg tablets (800 mg) under fasted conditions. The bioavailability of metaxalone was significantly higher in females compared to males as evidenced by $C_{max}$ (2115 ng/mL *versus* 1385 ng/mL) and $AUC_{\infty}$ (17884 ng.h/mL *versus* 10328 ng.h/mL). The mean half-life was 11.1 hours in females and 7.6 hours in males. The apparent volume of distribution of metaxalone was approximately 22% higher in males than in females, but not significantly different when adjusted for body weight. Similar findings were also seen when the previously described combined dataset was used in the analysis.

***Hepatic/Renal Insufficiency:*** The impact of hepatic and renal disease on the pharmacokinetics of metaxalone has not been determined. In the absence of such information, metaxalone tablets should be used with caution in patients with hepatic and/or renal impairment.

## INDICATIONS AND USAGE

Metaxalone tablets are indicated as an adjunct to rest, physical therapy and other measures for the relief of discomforts associated with acute, painful musculoskeletal conditions. The mode of action of this drug has not been clearly identified, but may be related to its sedative properties. Metaxalone does not directly relax tense skeletal muscles in man.

## CONTRAINDICATIONS

Known hypersensitivity to any components of this product.

Known tendency to drug induced, hemolytic or other anemias.

Significantly impaired renal or hepatic function.

## WARNINGS

Metaxalone tablets may enhance the effects of alcohol and other CNS depressants.

## PRECAUTIONS

Metaxalone should be administered with great care to patients with pre-existing liver damage. Serial liver function studies should be performed in these patients.

False-positive Benedict's tests, due to an unknown reducing substance, have been noted. A glucose-specific test will differentiate findings.

Taking metaxalone tablets with food may enhance general CNS depression; elderly patients may be especially susceptible to this CNS effect. (See **CLINICAL PHARMACOLOGY, Pharmacokinetics** and **PRECAUTIONS, Information for Patients**).

***Information for Patients***

Metaxalone tablets may impair mental and/or physical abilities required for performance of hazardous tasks, such as operating machinery or driving a motor vehicle, especially when used with alcohol or other CNS depressants.

***Drug Interactions:*** The sedative effects of metaxalone and other CNS depressants (e.g., alcohol, benzodiazepines, opioids, tricyclic antidepressants) may be additive. Therefore, caution should be exercised with patients who take more than one of these CNS depressants simultaneously.

***Carcinogenesis, Mutagenesis, Impairment of Fertility:*** The carcinogenic potential of metaxalone has not been determined.

***Pregnancy:*** Reproduction studies in rats have not revealed evidence of impaired fertility or harm to the fetus due to metaxalone. Post marketing experience has not revealed evidence of fetal injury, but such experience cannot exclude the possibility of infrequent or subtle damage to the human fetus. Safe use of metaxalone has not been established with regard to possible adverse effects upon fetal development. Therefore, metaxalone tablets should not be used in women who are or may become pregnant and particularly during early pregnancy unless in the judgement of the physician the potential benefits outweigh the possible hazards.

***Nursing Mothers:*** It is not known whether this drug is secreted in human milk. As a general rule, nursing should not be undertaken while a patient is on a drug since many drugs are excreted in human milk.

***Pediatric Use:*** Safety and effectiveness in children 12 years of age and below have not been established.

## ADVERSE REACTIONS

The most frequent reactions to metaxalone include:

***CNS:*** drowsiness, dizziness, headache and nervousness or "irritability";

***Digestive:*** nausea, vomiting, gastrointestinal upset.

Other adverse reactions are;

***Immune System:*** hypersensitivity reaction, rash with or without pruritus;

***Hematologic:*** leukopenia, hemolytic anemia;

***Hepatobiliary:*** jaundice.

Though rare, anaphylactoid reactions have been reported with metaxalone.

## OVERDOSAGE

Deaths by deliberate or accidental overdose have occurred with metaxalone, particularly in combination with antidepressants and have been reported with this class of drug in combination with alcohol.

When determining the $LD_{50}$ in rats and mice, progressive sedation, hypnosis and finally respiratory failure were noted as the dosage increased. In dogs, no $LD_{50}$ could be determined as the higher doses produced an emetic action in 15 to 30 minutes.

***Treatment***

Gastric lavage and supportive therapy. Consultation with a regional poison control center is recommended.

## DOSAGE AND ADMINISTRATION

The recommended dose for adults and children over 12 years of age is one 800 mg tablet three to four times a day.

## HOW SUPPLIED

Metaxalone Tablets are supplied as follows:

**800 mg Tablets:** rose-colored, capsule-shaped tablets, debossed "Ⓐ 448" on one side and scored on the other side and supplied as:

NDC 0185-0448-01 Bottles of 100.
NDC 0185-0448-10 Bottles of 1000.

Dispense contents in a tight, light-resistant container as defined in the USP with a child-resistant closure, as required.

Store at 20° to 25°C (68° to 77°F) [see USP Controlled Room Temperature].

**To report SUSPECTED ADVERSE REACTIONS, contact Sandoz Inc. at 1-800-525-8747 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.**

Sandoz Inc.
Princeton, NJ 08540

OS7750
Issued 10/09

MF0446IS10/09
MG #17653

# EXHIBIT D

**FLUVOXAMINE MALEATE - fluvoxamine maleate tablet, film coated**
Sandoz Inc.

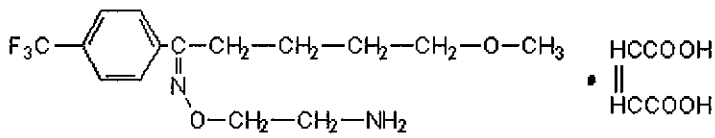
**Rx only**

**Suicidality and Antidepressant DrugsAntidepressants increased the risk compared to placebo of suicidal thinking and behavior (suicidality) in children, adolescents, and young adults in short-term studies of major depressive disorder (MDD) and other psychiatric disorders. Anyone considering the use of fluvoxamine maleate or any other antidepressant in a child, adolescent, or young adult must balance this risk with the clinical need. Short-term studies did not show an increase in the risk of suicidality with antidepressants compared to placebo in adults beyond age 24; there was a reduction in risk with antidepressants compared to placebo in adults aged 65 and older. Depression and certain other psychiatric disorders are themselves associated with increases in the risk of suicide. Patients of all ages who are started on antidepressant therapy should be monitored appropriately and observed closely for clinical worsening, suicidality, or unusual changes in behavior. Families and caregivers should be advised of the need for close observation and communication with the prescriber. Fluvoxamine is not approved for use in pediatric patients except for patients with obsessive compulsive disorder (OCD). (See WARNINGS and PRECAUTIONS, Pediatric Use.) (See WARNINGS, Clinical Worsening and Suicide Risk, PRECAUTIONS, Information for Patients.)**

## DESCRIPTION

Fluvoxamine maleate is a selective serotonin (5-HT) reuptake inhibitor (SSRI) belonging to a new chemical series, the 2-aminoethyl oxime ethers of aralkylketones. It is chemically unrelated to other SSRIs and clomipramine. It is chemically designated as 5-methoxy-4'-(trifluoromethyl)valerophenone-(E)-O-(2-aminoethyl)oxime maleate (1:1) and has the molecular formula $C_{15}H_{21}O_2N_2F_3 \cdot C_4H_4O_4$. Its molecular weight is 434.4.

The structural formula is:



$C_{15}H_{21}O_2N_2F_3 \cdot C_4H_4O_4$ M.W. 434.4

Fluvoxamine maleate is a white or off white, odorless, crystalline powder which is sparingly soluble in water, freely soluble in ethanol and chloroform and practically insoluble in diethyl ether.

Fluvoxamine Maleate Tablets are available in 25 mg, 50 mg and 100 mg strengths for oral administration. In addition to the active ingredient, fluvoxamine maleate, each tablet contains the following inactive ingredients: carnauba wax, corn starch, hypromellose (3cP), hypromellose (6cP), magnesium stearate, mannitol, methylcellulose, polyethylene glycol, polysorbate 80, pregelatinized starch, purified water, sodium starch glycolate, titanium dioxide, and yellow iron oxide. The 100 mg tablets also contain red iron oxide.

## CLINICAL PHARMACOLOGY

### Pharmacodynamics

The mechanism of action of fluvoxamine maleate in Obsessive Compulsive Disorder is presumed to be linked to its specific serotonin reuptake inhibition in brain neurons. In preclinical studies, it was found that fluvoxamine inhibited neuronal uptake of serotonin. In *in vitro* studies fluvoxamine maleate had no significant affinity for histaminergic, alpha or beta adrenergic, muscarinic, or dopaminergic receptors. Antagonism of some of these receptors is thought to be associated with various sedative, cardiovascular, anticholinergic, and extrapyramidal effects of some psychotropic drugs.

### Pharmacokinetics

Bioavailability

The absolute bioavailability of fluvoxamine maleate is 53%. Oral bioavailability is not significantly affected by food.

In a dose proportionality study involving fluvoxamine maleate at 100 mg/day, 200 mg/day and 300 mg/day for 10 consecutive days in 30 normal volunteers, steady state was achieved after about a week of dosing. Maximum plasma concentrations at steady state occurred within 3 to 8 hours of dosing and reached concentrations averaging 88 ng/mL, 283 ng/mL and 546 ng/mL, respectively. Thus, fluvoxamine had nonlinear pharmacokinetics over this dose range, i.e., higher doses of fluvoxamine maleate produced disproportionately higher concentrations than predicted from the lower dose.

Distribution/Protein Binding

The mean apparent volume of distribution for fluvoxamine is approximately 25 L/kg, suggesting extensive tissue distribution. Approximately 80% of fluvoxamine is bound to plasma protein, mostly albumin, over a concentration range of 20 ng/mL to 2000 ng/mL.

Metabolism

Fluvoxamine maleate is extensively metabolized by the liver; the main metabolic routes are oxidative demethylation and deamination. Nine metabolites were identified following a 5 mg radiolabelled dose of fluvoxamine maleate, constituting approximately 85% of the urinary excretion products of fluvoxamine. The main human metabolite was fluvoxamine acid which, together with its N-acetylated analog, accounted for about 60% of the urinary excretion products. A third metabolite, fluvoxethanol, formed by oxidative deamination, accounted for about 10%. Fluvoxamine acid and fluvoxethanol were tested in an *in vitro* assay of serotonin and norepinephrine reuptake inhibition in rats; they were inactive except for a weak effect of the former metabolite on inhibition of serotonin uptake (1 to 2 orders of magnitude less potent than the parent compound). Approximately 2% of fluvoxamine was excreted in urine unchanged. (See **PRECAUTIONS,**Drug Interactions.)

Elimination

Following a $^{14}C$-labelled oral dose of fluvoxamine maleate (5 mg), an average of 94% of drug-related products was recovered in the urine within 71 hours. The mean plasma half-life of fluvoxamine at steady state after multiple oral doses of 100 mg/day in healthy, young volunteers was 15.6 hours.

Elderly Subjects

In a study of fluvoxamine maleate tablets at 50 mg and 100 mg comparing elderly (ages 66 to 73) and young subjects (ages 19 to 35), mean maximum plasma concentrations in the elderly were 40% higher. The multiple dose elimination half-life of fluvoxamine was 17.4 and 25.9 hours in the elderly compared to 13.6 and 15.6 hours in the young subjects at steady state for 50 mg and 100 mg doses, respectively.

In elderly patients, the clearance of fluvoxamine was reduced by about 50% and, therefore, fluvoxamine maleate tablets should be slowly titrated during initiation of therapy.

Pediatric Subjects

The multiple-dose pharmacokinetics of fluvoxamine were determined in male and female children (ages 6 to 11) and adolescents (ages 12 to 17). Steady-state plasma fluvoxamine concentrations were 2- to 3-fold higher in children than in adolescents. AUC and $C_{max}$ in children were 1.5- to 2.7-fold higher than that in adolescents (see table below). As in adults, both children and adolescents exhibited nonlinear multiple-dose pharmacokinetics. Female children showed significantly higher AUC (0 to 12) and $C_{max}$ compared to male children and, therefore, lower doses of fluvoxamine maleate tablets may produce therapeutic benefit (see table below). No gender differences were observed in adolescents. Steady-state plasma fluvoxamine concentrations were similar in adults and adolescents at a dose of 300 mg/day, indicating that fluvoxamine exposure was similar in these two populations (see table below). Dose adjustment in adolescents (up to the adult maximum dose of 300 mg) may be indicated to achieve therapeutic benefit.

Comparison of Mean (SD) fluvoxamine pharmacokinetic parameters between children, adolescents and adults.

| Pharmacokinetic Parameter (body weight corrected) | Dose = 200 mg/day (100 mg BID) | | Dose = 300 mg/day (150 mg BID) | |
|---|---|---|---|---|
| | Children (n=10) | Adolescent (n=17) | Adolescents (n=13) | Adults (n=16) |
| AUC 0-12 (ng.h/mL/kg) | 155.1 (160.9) | 43.9 (27.9) | 69.6 (46.6) | 59.4 (40.9) |
| $C_{max}$ (ng/mL/kg) | 14.8 (14.9) | 4.2 (2.6) | 6.7 (4.2) | 5.7 (3.9) |
| $C_{min}$ (ng/mL/kg) | 11.0 (11.9) | 2.9 (2.0) | 4.8 (3.8) | 4.6 (3.2) |

Comparison of Mean (SD) fluvoxamine pharmacokinetic parameters between male and female children (6 to 11 years)

| Pharmacokinetic Parameter | Dose = 200 mg/day (100 mg BID) | |
|---|---|---|
| (body weight corrected) | Male Children (n=7) | Female children (n=3) |
| AUC 0-12 (ng.h/mL/kg) | 95.8 (83.9) | 293.5 (233.0) |
| $C_{max}$ (ng/mL/kg) | 9.1 (7.6) | 28.1 (21.1) |
| $C_{min}$ (ng/mL/kg) | 6.6 (6.1) | 21.2 (17.6) |

Hepatic and Renal Disease

A cross study comparison (healthy subjects vs. patients with hepatic dysfunction) suggested a 30% decrease in fluvoxamine clearance in association with hepatic dysfunction. The mean minimum plasma concentrations in renally impaired patients (creatinine clearance

of 5 mL/min to 45 mL/min) after 4 and 6 weeks of treatment (50 mg BID, N=13) were comparable to each other, suggesting no accumulation of fluvoxamine in these patients. (See **PRECAUTIONS** - ***Use in Patients with Concomitant Illness.***)

**Clinical Trials**

Adult OCD Studies

The effectiveness of fluvoxamine maleate tablets for the treatment of Obsessive Compulsive Disorder (OCD) was demonstrated in two 10-week multicenter, parallel group studies of adult outpatients. Patients in these trials were titrated to a total daily fluvoxamine maleate dose of 150 mg/day over the first two weeks of the trial, following which the dose was adjusted within a range of 100 mg/day to 300 mg/day (on a BID schedule), on the basis of response and tolerance. Patients in these studies had moderate to severe OCD (DSM-III-R), with mean baseline ratings on the Yale-Brown Obsessive Compulsive Scale (Y-BOCS), total score of 23. Patients receiving fluvoxamine maleate experienced mean reductions of approximately 4 to 5 units on the Y-BOCS total score, compared to a 2 unit reduction for placebo patients.

The following table provides the outcome classification by treatment group on the Global Improvement item of the Clinical Global Impressions (CGI) scale for both studies combined.

**OUTCOME CLASSIFICATION (%) ON CGI-GLOBAL IMPROVEMENT ITEM FOR COMPLETERS IN POOL OF TWO ADULT OCD STUDIES**

| Outcome Classification | Fluvoxamine (N=120) | Placebo (N=134) |
|---|---|---|
| Very Much Improved | 13% | 2% |
| Much Improved | 30% | 10% |
| Minimally Improved | 22% | 32% |
| No Change | 31% | 51% |
| Worse | 4% | 6% |

Exploratory analyses for age and gender effects on outcomes did not suggest any differential responsiveness on the basis of age or sex.

Pediatric OCD Study

The effectiveness of fluvoxamine maleate tablets for the treatment of OCD was also demonstrated in a 10-week multicenter, parallel group study in a pediatric outpatient population (children and adolescents, ages 8 to 17). Patients in this study were titrated to a total daily fluvoxamine dose of approximately 100 mg/day over the first two weeks of the trial, following which the dose was adjusted within a range of 50 mg/day to 200 mg/day (on a BID schedule) on the basis of response and tolerance. All patients had moderate-to-severe OCD (DSM-III-R) with mean baseline ratings on the children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) total score of 24. Patients receiving fluvoxamine maleate experienced mean reductions of approximately 6 units on the CY-BOCS total score, compared to a three-unit reduction for placebo patients.

The following table provides the outcome classification by treatment group on the Global Improvement item of the Clinical Global Impression (CGI) scale for the pediatric study.

**OUTCOME CLASSIFICATION (%) ON CGI-GLOBAL IMPROVEMENT ITEM FOR COMPLETERS IN PEDIATRIC STUDY**

| Outcome Classification | Fluvoxamine (N=38) | Placebo (N=36) |
|---|---|---|
| Very Much Improved | 21% | 11% |
| Much Improved | 18% | 17% |
| Minimally Improved | 37% | 22% |
| No Change | 16% | 44% |
| Worse | 8% | 6% |

Post hoc exploratory analyses for gender effects on outcomes did not suggest any differential responsiveness on the basis of gender. Further exploratory analyses revealed a prominent treatment effect in the 8 to 11 age group and essentially no effect in the 12 to 17 age group. While the significance of these results is not clear, the 2 to 3 fold higher steady state plasma fluvoxamine concentrations in children compared to adolescents (see **Pharmacokinetics**) is suggestive that decreased exposure in adolescents may have been a factor, and dose adjustment in adolescents (up to the adult maximum dose of 300 mg) may be indicated to achieve therapeutic benefit.

**INDICATIONS AND USAGE**

Fluvoxamine maleate tablets are indicated for the treatment of obsessions and compulsions in patients with Obsessive Compulsive Disorder (OCD), as defined in the DSM-III-R. The obsessions or compulsions cause marked distress, are time-consuming, or significantly interfere with social or occupational functioning.

The efficacy of fluvoxamine maleate tablets was established in three 10-week trials with obsessive compulsive outpatients with the diagnosis of Obsessive Compulsive Disorder as defined in DSM-III-R. (See Clinical Trials under **CLINICAL PHARMACOLOGY**.)

Obsessive Compulsive Disorder is characterized by recurrent and persistent ideas, thoughts, impulses or images (obsessions) that are ego-dystonic and/or repetitive, purposeful, and intentional behaviors (compulsions) that are recognized by the person as excessive or unreasonable.

The effectiveness of fluvoxamine maleate tablets for long-term use, i.e., for more than 10 weeks, has not been systematically evaluated in placebo-controlled trials. Therefore, the physician who elects to use fluvoxamine maleate tablets for extended periods should periodically re-evaluate the long-term usefulness of the drug for the individual patient. (See **DOSAGE AND ADMINISTRATION.**)

**CONTRAINDICATIONS**

Co-administration of thioridazine, terfenadine, astemizole, cisapride, pimozide, alosetron or tizanidine with fluvoxamine maleate is contraindicated (see WARNINGS, PRECAUTIONS, and Lotronex™ (alosetron) package insert). Fluvoxamine maleate tablets are contraindicated in patients with a history of hypersensitivity to fluvoxamine maleate.

**WARNINGS**

**Potential for Interaction with Monoamine Oxidase Inhibitors**

**In patients receiving another serotonin reuptake inhibitor drug in combination with monoamine oxidase inhibitors (MAOI), there have been reports of serious, sometimes fatal, reactions including hyperthermia, rigidity, myoclonus, autonomic instability with possible rapid fluctuations of vital signs, and mental status changes that include extreme agitation progressing to delirium and coma. These reactions have also been reported in patients who have discontinued that drug and have been started on a MAOI. Some cases presented with features resembling neuroleptic malignant syndrome. Therefore, it is recommended that fluvoxamine maleate tablets not be used in combination with a MAOI, or within 14 days of discontinuing treatment with a MAOI. After stopping fluvoxamine maleate tablets, at least 2 weeks should be allowed before starting a MAOI.**

**Potential Interaction with Thioridazine**

**The effect of fluvoxamine (25 mg BID for one week) on thioridazine steady-state concentrations was evaluated in 10 male inpatients with schizophrenia. Concentrations of thioridazine and its two active metabolites, mesoridazine and sulforidazine, increased threefold following coadministration of fluvoxamine.**

**Thioridazine administration produces a dose-related prolongation of the QTc interval, which is associated with serious ventricular arrhythmias, such as torsade de pointes-type arrhythmias, and sudden death. It is likely that this experience underestimates the degree of risk that might occur with higher doses of thioridazine. Moreover, the effect of fluvoxamine may be even more pronounced when it is administered at higher doses.**

**Therefore, fluvoxamine and thioridazine should not be co-administered (see CONTRAINDICATIONS and PRECAUTIONS).**

**Potential Terfenadine, Astemizole, Cisapride, and Pimozide Interactions**

**Terfenadine, astemizole, cisapride, and pimozide are all metabolized by the CYP3A4 isozyme, and it has been demonstrated that ketoconazole, a potent inhibitor of 3A4, blocks the metabolism of these drugs, resulting in increased plasma concentrations of parent drug. Increased plasma concentrations of terfenadine, astemizole, cisapride, and pimozide cause QT prolongation and have been associated with torsades de pointes-type ventricular tachycardia, sometimes fatal. As noted below, a substantial pharmacokinetic interaction has been observed for fluvoxamine in combination with alprazolam, a drug that is known to be metabolized by the 3A4 isozyme. Although it has not been definitively demonstrated that fluvoxamine is a potent 3A4 inhibitor, it is likely to be, given the substantial interaction of fluvoxamine with alprazolam. Consequently, it is recommended that fluvoxamine not be used in combination with either terfenadine, astemizole, cisapride, or pimozide (seeCONTRAINDICATIONS and PRECAUTIONS.)**

**Potential Tizanidine Interaction**

**Fluvoxamine is a potent inhibitor of CYP1A2 and tizanidine is a CYP1A2 substrate. The effect of fluvoxamine (100 mg daily for 4 days) on the pharmacokinetics and pharmacodynamics of a single 4 mg dose of tizanidine has been studied in 10 healthy subjects. Tizanidine $C_{max}$ was increased approximately 12-fold (range 5-fold to 32-fold), elimination half-life was increased by almost 3-fold, and AUC increased 33-fold (range 14-fold to 103-fold). The mean maximal effect on blood pressure was a 35 mm Hg decrease in systolic blood pressure, a 20 mm Hg decrease in diastolic blood pressure, and a 4 beat/min decrease**

**in heart rate. Drowsiness was significantly increased and performance on a psychomotor task was significantly impaired. Fluvoxamine and tizanidine should not be used together. (See CONTRAINDICATIONS and PRECAUTIONS.)**

**Potential Alosetron Interaction**

**Fluvoxamine, an inhibitor of several CYP isozymes, has been shown to increase mean alosetron plasma concentrations (AUC) approximately 6-fold and prolonged the half-life by approximately 3-fold. Consequently, it is recommended that fluvoxamine not be used in combination with alosetron (see CONTRAINDICATIONS, PRECAUTIONS and Lotronex™ (alosetron) package insert).**

**Clinical Worsening and Suicide Risk**

Patients with major depressive disorder (MDD), both adult and pediatric, may experience worsening of their depression and/or the emergence of suicidal ideation and behavior (suicidality) or unusual changes in behavior, whether or not they are taking antidepressant medications, and this risk may persist until significant remission occurs. Suicide is a known risk of depression and certain other psychiatric disorders, and these disorders themselves are the strongest predictors of suicide. There has been a long-standing concern, however, that antidepressants may have a role in inducing worsening of depression and the emergence of suicidality in certain patients during the early phases of treatment. Pooled analyses of short-term placebo-controlled trials of antidepressant drugs (SSRIs and others) showed that these drugs increase the risk of suicidal thinking and behavior (suicidality) in children, adolescents, and young adults (ages 18-24) with major depressive disorder (MDD) and other psychiatric disorders. Short-term studies did not show an increase in the risk of suicidality with antidepressants compared to placebo in adults beyond age 24; there was a reduction with antidepressants compared to placebo in adults aged 65 and older. The pooled analyses of placebo-controlled trials in children and adolescents with MDD, obsessive compulsive disorder (OCD), or other psychiatric disorders included a total of 24 short-term trials of 9 antidepressant drugs in over 4400 patients. The pooled analyses of placebo-controlled trials in adults with MDD or other psychiatric disorders included a total of 295 short-term trials (median duration of 2 months) of 11 antidepressant drugs in over 77,000 patients. There was considerable variation in risk of suicidality among drugs, but a tendency toward an increase in the younger patients for almost all drugs studied. There were differences in absolute risk of suicidality across the different indications, with the highest incidence in MDD. The risk differences (drug vs placebo), however, were relatively stable within age strata and across indications. These risk differences (drug-placebo difference in the number of cases of suicidality per 1,000 patients treated) are provided in Table 1.

Table 1

| **Age Range** | **Drug-Placebo Difference in Number of Cases of Suicidality Per 1000 Patients Treated** |
|---|---|
| | Increases Compared to Placebo |
| <18 | 14 additional cases |
| 18-24 | 5 additional cases |
| | Decreases Compared to Placebo |
| 25-64 | 1 fewer case |
| ≥65 | 6 fewer cases |

No suicides occurred in any of the pediatric trials. There were suicides in the adult trials, but the number was not sufficient to reach any conclusion about drug effect on suicide.

It is unknown whether the suicidality risk extends to longer-term use, i.e., beyond several months. However, there is substantial evidence from placebo-controlled maintenance trials in adults with depression that the use of antidepressants can delay the recurrence of depression.

**All patients being treated with antidepressants for any indication should be monitored appropriately and observed closely for clinical worsening, suicidality, and unusual changes in behavior, especially during the initial few months of a course of drug therapy, or at times of dose changes, either increases or decreases.**

The following symptoms, anxiety, agitation, panic attacks, insomnia, irritability, hostility, aggressiveness, impulsivity, akathisia (psychomotor restlessness), hypomania, and mania, have been reported in adult and pediatric patients being treated with antidepressants for major depressive disorder as well as for other indications, both psychiatric and nonpsychiatric. Although a causal link between the emergence of such symptoms and either the worsening of depression and/or the emergence of suicidal impulses has not been established, there is concern that such symptoms may represent precursors to emerging suicidality.

Consideration should be given to changing the therapeutic regimen, including possibly discontinuing the medication, in patients whose depression is persistently worse, or who are experiencing emergent suicidality or symptoms that might be precursors to worsening depression or suicidality, especially if these symptoms are severe, abrupt in onset, or were not part of the patient's presenting symptoms.

If the decision has been made to discontinue treatment, medication should be tapered, as rapidly as is feasible, but with recognition that abrupt discontinuation can be associated with certain symptoms (see PRECAUTIONS and **DOSAGE AND**

**ADMINISTRATION, Discontinuation of Treatment with Fluvoxamine Maleate Tablets**, for a description of the risks of discontinuation of fluvoxamine maleate).

**Families and caregivers of patients being treated with antidepressants for major depressive disorder or other indications, both psychiatric and nonpsychiatric, should be alerted about the need to monitor patients for the emergence of agitation, irritability, unusual changes in behavior, and the other symptoms described above, as well as the emergence of suicidality, and to report such symptoms immediately to health care providers. Such monitoring should include daily observation by families and caregivers.** Prescriptions for fluvoxamine maleate tablets should be written for the smallest quantity of tablets consistent with good patient management, in order to reduce the risk of overdose.

Screening Patients for Bipolar Disorder

A major depressive episode may be the initial presentation of bipolar disorder. It is generally believed (though not established in controlled trials) that treating such an episode with an antidepressant alone may increase the likelihood of precipitation of a mixed/manic episode in patients at risk for bipolar disorder. Whether any of the symptoms described above represent such a conversion is unknown. However, prior to initiating treatment with an antidepressant, patients with depressive symptoms should be adequately screened to determine if they are at risk for bipolar disorder; such screening should include a detailed psychiatric history, including a family history of suicide, bipolar disorder, and depression. It should be noted that fluvoxamine maleate is not approved for use in treating bipolar depression.

**Other Potentially Important Drug Interactions**

(Also see **PRECAUTIONS**, Drug Interactions)

Benzodiazepines

Benzodiazepines metabolized by hepatic oxidation (e.g., alprazolam, midazolam, triazolam, etc.) should be used with caution because the clearance of these drugs is likely to be reduced by fluvoxamine. The clearance of benzodiazepines metabolized by glucuronidation (e.g., lorazepam, oxazepam, temazepam) is unlikely to be affected by fluvoxamine.

Alprazolam

When fluvoxamine maleate (100 mg QD) and alprazolam (1 mg QID) were co-administered to steady state, plasma concentrations and other pharmacokinetic parameters (AUC, Cmax, $T_{1/2}$) of alprazolam were approximately twice those observed when alprazolam was administered alone; oral clearance was reduced by about 50%. The elevated plasma alprazolam concentrations resulted in decreased psychomotor performance and memory. This interaction, which has not been investigated using higher doses of fluvoxamine, may be more pronounced if a 300 mg daily dose is co-administered, particularly since fluvoxamine exhibits non-linear pharmacokinetics over the dosage range 100 mg to 300 mg. If alprazolam is co-administered with fluvoxamine maleate tablets, the initial alprazolam dosage should be at least halved and titration to the lowest effective dose is recommended. No dosage adjustment is required for fluvoxamine maleate tablets.

Diazepam

The co-administration of fluvoxamine maleate tablets and diazepam is generally not advisable. Because fluvoxamine reduces the clearance of both diazepam and its active metabolite, N-desmethyldiazepam, there is a strong likelihood of substantial accumulation of both species during chronic co-administration.

Evidence supporting the conclusion that it is inadvisable to co-administer fluvoxamine and diazepam is derived from a study in which healthy volunteers taking 150 mg/day of fluvoxamine were administered a single oral dose of 10 mg of diazepam. In these subjects (N=8), the clearance of diazepam was reduced by 65% and that of N-desmethyldiazepam to a level that was too low to measure over the course of the 2 week long study.

It is likely that this experience significantly underestimates the degree of accumulation that might occur with repeated diazepam administration. Moreover, as noted with alprazolam, the effect of fluvoxamine may even be more pronounced when it is administered at higher doses.

Accordingly, diazepam and fluvoxamine should not ordinarily be co-administered.

Mexiletine

The effect of steady state fluvoxamine (50 mg BID for 7 days) on the single dose pharmacokinetics of mexiletine (200 mg) was evaluated in 6 healthy Japanese males. The clearance of mexiletine was reduced by 38% following co-administration with fluvoxamine compared to mexiletine alone. If fluvoxamine and mexiletine are co-administered, serum mexiletine levels should be monitored.

Serotonin Syndrome

The development of a potentially life-threatening serotonin syndrome may occur with fluvoxamine treatment, particularly with concomitant use of serotonergic drugs (including triptans) and with drugs which impair metabolism of serotonin (including MAOIs).

Serotonin syndrome symptoms may include mental status changes (e.g., agitation, hallucinations, coma), autonomic instability (e.g., tachycardia, labile blood pressure, hyperthermia), neuromuscular aberrations (e.g., hyperreflexia, incoordination) and/or gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea).

The concomitant use of fluvoxamine with MAOIs intended to treat depression is contraindicated (see CONTRAINDICATIONS and **WARNINGS**, Potential for Interaction with Monoamine Oxidase Inhibitors.)

If concomitant treatment of fluvoxamine with a 5-hydroxytryptamine receptor agonist (triptan) is clinically warranted, careful observation of the patient is advised, particularly during treatment initiation and dose increases (see **PRECAUTIONS, Drug Interactions).**

The concomitant use of fluvoxamine with serotonin precursors (such as tryptophan) is not recommended (see **PRECAUTIONS, Drug Interactions).**

Neuroleptic Malignant Syndrome (NMS) or NMS-Like Reactions
Rare instances of neuroleptic malignant syndrome (NMS) or NMS-like reactions have been reported when a selective serotonin reuptake inhibitor (SSRI) drug, such as Fluvoxamine Maleate Tablets, or a serotonin-norepinephrine reuptake inhibitor (SNRI) was added to antipsychotic drug therapy. Additionally, a small number of such cases have been reported with SSRI's and SNRI's in the absence of antipsychotic coadministration. These serious and sometimes fatal events can include hyperthermia, muscle rigidity, autonomic instability with possible rapid fluctuation of vital signs, and mental status changes. It is uncertain whether these cases are serotonin syndrome which, in its most severe form, can resemble neuroleptic malignant syndrome. As these events may result in potentially life-threatening conditions, patients should be monitored for the emergence of NMS-like signs and symptoms, especially if Fluvoxamine Maleate Tablets and an antipsychotic drug are taken concurrently. Treatment with Fluvoxamine Maleate Tablets and any concomitant antipsychotic agent should be discontinued immediately if such events occur and supportive symptomatic treatment should be initiated.

Theophylline
The effect of steady-state fluvoxamine (50 mg BID) on the pharmacokinetics of a single dose of theophylline (375 mg as 442 mg aminophylline) was evaluated in 12 healthy nonsmoking, male volunteers. The clearance of theophylline was decreased approximately three-fold. Therefore, if theophylline is co-administered with fluvoxamine maleate, its dose should be reduced to one third of the usual daily maintenance dose and plasma concentrations of theophylline should be monitored. No dosage adjustment is required for fluvoxamine maleate tablets.

Warfarin
When fluvoxamine maleate (50 mg TID) was administered concomitantly with warfarin for two weeks, warfarin plasma concentrations increased by 98% and prothrombin times were prolonged. Thus patients receiving oral anticoagulants and fluvoxamine maleate tablets should have their prothrombin time monitored and their anticoagulant dose adjusted accordingly. No dosage adjustment is required for fluvoxamine maleate tablets.

**PRECAUTIONS**

**General**

Discontinuation of Treatment with Fluvoxamine Maleate Tablets
During marketing of fluvoxamine maleate tablets and other SSRIs and SNRIs (Serotonin and Norepinephrine Reuptake inhibitors), there have been spontaneous reports of adverse events occurring upon discontinuation of these drugs, particularly when abrupt, including the following: dysphoric mood, irritability, agitation, dizziness, sensory disturbances (e.g., paresthesias, such as electric shock sensations), anxiety, confusion, headache, lethargy, emotional lability, insomnia, and hypomania. While these events are generally self-limiting, there have been reports of serious discontinuation symptoms. Patients should be monitored for these symptoms when discontinuing treatment with fluvoxamine maleate tablets. A gradual reduction in the dose rather than abrupt cessation is recommended whenever possible. If intolerable symptoms occur following a decrease in the dose or upon discontinuation of treatment, then resuming the previously prescribed dose may be considered. Subsequently, the physician may continue decreasing the dose but at a more gradual rate (see DOSAGE AND ADMINISTRATION).

Abnormal Bleeding
Published case reports have documented the occurrence of bleeding episodes in patients treated with psychotropic drugs that interfere with serotonin reuptake. Subsequent epidemiological studies, both of the case-control and cohort design, have demonstrated an association between use of psychotropic drugs that interfere with serotonin reuptake and the occurrence of upper gastrointestinal bleeding. In two studies, concurrent use of a nonsteroidal anti-inflammatory drug (NSAID) or aspirin potentiated the risk of bleeding (see Drug Interactions). Although these studies focused on upper gastrointestinal bleeding, there is reason to believe that bleeding at

other sites may be similarly potentiated. Patients should be cautioned regarding the risk of bleeding associated with the concomitant use of fluvoxamine with NSAIDs, aspirin, or other drugs that affect coagulation.

Activation of Mania/Hypomania
During premarketing studies involving primarily depressed patients, hypomania or mania occurred in approximately 1% of patients treated with fluvoxamine. In a ten week pediatric OCD study, 2 out of 57 patients (4%) treated with fluvoxamine experienced manic reactions, compared to none of 63 placebo patients. Activation of mania/hypomania has also been reported in a small proportion of patients with major affective disorder who were treated with other marketed antidepressants. As with all antidepressants, fluvoxamine maleate tablets should be used cautiously in patients with a history of mania.

Seizures
During premarketing studies, seizures were reported in 0.2% of fluvoxamine-treated patients. Fluvoxamine maleate tablets should be used cautiously in patients with a history of seizures. It should be discontinued in any patient who develops seizures.

Hyponatremia
Hyponatremia may occur as a result of treatment with SSRIs and SNRIs, including fluvoxamine. In many cases, this hyponatremia appears to be the result of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Cases with serum sodium lower than 110 mmol/L have been reported. Elderly patients may be at greater risk of developing hyponatremia with SSRIs and SNRIs. Also, patients taking diuretics or who are otherwise volume depleted may be at greater risk (see Geriatric Use). Discontinuation of fluvoxamine should be considered in patients with symptomatic hyponatremia and appropriate medical intervention should be instituted.

Signs and symptoms of hyponatremia include headache, difficulty concentrating, memory impairment, confusion, weakness, and unsteadiness, which may lead to falls. Signs and symptoms associated with more severe and/or acute cases have included hallucination, syncope, seizure, coma, respiratory arrest, and death.

Use in Patients with Concomitant Illness
Closely monitored clinical experience with fluvoxamine maleate tablets in patients with concomitant systemic illness is limited. Caution is advised in administering fluvoxamine maleate tablets to patients with diseases or conditions that could affect hemodynamic responses or metabolism.

Fluvoxamine maleate tablets have not been evaluated or used to any appreciable extent in patients with a recent history of myocardial infarction or unstable heart disease. Patients with these diagnoses were systematically excluded from many clinical studies during the product's premarketing testing. Evaluation of the electrocardiograms for patients with depression or OCD who participated in premarketing studies revealed no differences between fluvoxamine and placebo in the emergence of clinically important ECG changes.

In patients with liver dysfunction, fluvoxamine clearance was decreased by approximately 30%. Fluvoxamine maleate tablets should be slowly titrated in patients with liver dysfunction during the initiation of treatment.

**Information for Patients**
Prescribers or other health professionals should inform patients, their families, and their caregivers about the benefits and risks associated with treatment with fluvoxamine maleate and should counsel them in its appropriate use. A patient Medication Guide about "Antidepressant Medicines, Depression and other Serious Mental Illness, and Suicidal Thoughts or Actions" is available for fluvoxamine maleate. The prescriber or health professional should instruct patients, their families, and their caregivers to read the Medication Guide and should assist them in understanding its contents. Patients should be given the opportunity to discuss the contents of the Medication Guide and to obtain answers to any questions they may have. The complete text of the Medication Guide is reprinted at the end of this document.
Patients should be advised of the following issues and asked to alert their prescriber if these occur while taking fluvoxamine maleate.
Patients should be cautioned about the risk of serotonin syndrome with the concomitant use of fluvoxamine and triptans, tramadol or other serotonergic agents.

Clinical Worsening and Suicide Risk
Patients, their families, and their caregivers should be encouraged to be alert to the emergence of anxiety, agitation, panic attacks, insomnia, irritability, hostility, aggressiveness, impulsivity, akathisia (psychomotor restlessness), hypomania, mania, other unusual changes in behavior, worsening of depression, and suicidal ideation, especially early during antidepressant treatment and when the dose is adjusted up or down. Families and caregivers of patients should be advised to look for the emergence of such symptoms on a day-to-day basis, since changes may be abrupt. Such symptoms should be reported to the patient's prescriber or health professional, especially if they are severe, abrupt in onset, or were not part of the patient's presenting symptoms. Symptoms such as these may be associated with an increased risk for suicidal thinking and behavior and indicate a need for very close monitoring and possibly changes in the medication.

Interference with Cognitive or Motor Performance
Since any psychoactive drug may impair judgment, thinking, or motor skills, patients should be cautioned about operating hazardous machinery, including automobiles, until they are certain that fluvoxamine maleate tablet therapy does not adversely affect their ability to engage in such activities.

Pregnancy
Patients should be advised to notify their physicians if they become pregnant or intend to become pregnant during therapy with fluvoxamine maleate tablets.

Nursing
Patients receiving fluvoxamine maleate tablets should be advised to notify their physicians if they are breast feeding an infant. (See **PRECAUTIONS, Nursing Mothers.)**

Concomitant Medication:
Patients should be advised to notify their physicians if they are taking, or plan to take, any prescription or over-the-counter drugs, since there is a potential for clinically important interactions with fluvoxamine maleate tablets. Patients should be cautioned about the concomitant use of fluvoxamine and NSAIDs, aspirin, or other drugs that affect coagulation since the combined use of psychotropic drugs that interfere with serotonin reuptake and these agents has been associated with an increased risk of bleeding.

Because of the potential for the increased risk of serious adverse reactions including severe lowering of blood pressure and sedation when fluvoxamine and tizanidine are used together, fluvoxamine should not be used with tizanidine.

Because of the potential for the increased risk of serious adverse reactions when fluvoxamine and alosetron are used together, fluvoxamine should not be used with Lotronex™ (alosetron).

Alcohol
As with other psychotropic medications, patients should be advised to avoid alcohol while taking fluvoxamine maleate tablets.

Allergic Reactions
Patients should be advised to notify their physicians if they develop a rash, hives, or a related allergic phenomenon during therapy with fluvoxamine maleate tablets.

**Laboratory Tests**
There are no specific laboratory tests recommended.

**Drug Interactions**

Potential Interactions with Drugs that Inhibit or are Metabolized by Cytochrome P450 Isozymes
Multiple hepatic cytochrome P450 (CYP450) enzymes are involved in the oxidative biotransformation of a large number of structurally different drugs and endogenous compounds. The available knowledge concerning the relationship of fluvoxamine and the CYP450 enzyme system has been obtained mostly from pharmacokinetic interaction studies conducted in healthy volunteers, but some preliminary *in vitro* data are also available. Based on a finding of substantial interactions of fluvoxamine with certain of these drugs (see later parts of this section and also WARNINGS for details) and limited *in vitro* data for the 3A4 isozyme, it appears that fluvoxamine inhibits the following isozymes that are known to be involved in the metabolism of the listed drugs:

| 1A2 | 2C9 | 3A4 | 2C19 |
|---|---|---|---|
| Warfarin | Warfarin | Alprazolam | Omeprazole |
| Theophylline | — | — | — |
| Propranolol | — | — | — |
| Tizanidine | — | — | — |

*In vitro* data suggest that fluvoxamine is a relatively weak inhibitor of the 2D6 isozyme.

Approximately 7% of the normal population has a genetic defect that leads to reduced levels of activity of the CYP2D6 isozyme. Such individuals have been referred to as "poor metabolizers" (PM) of drugs such as debrisoquin, dextromethorphan, and tricyclic antidepressants. While none of the drugs studied for drug interactions significantly affected the pharmacokinetics of fluvoxamine, an *in vivo* study of fluvoxamine single-dose pharmacokinetics in 13 PM subjects demonstrated altered pharmacokinetic properties compared to 16 "extensive metabolizers" (EM): mean $C_{max}$, AUC, and half-life were increased by 52%, 200%, and 62%, respectively, in the PM compared to the EM group. This suggests that fluvoxamine is metabolized, at least in part, by the 2D6 isozyme. Caution is

indicated in patients known to have reduced levels of CYP2D6 activity and those receiving concomitant drugs known to inhibit this isozyme (e.g. quinidine).

The metabolism of fluvoxamine has not been fully characterized and the effects of potent P450 isozyme inhibition, such as the ketoconazole inhibition of 3A4, on fluvoxamine metabolism have not been studied.

A clinically significant fluvoxamine interaction is possible with drugs having a narrow, therapeutic ratio such as terfenadine, astemizole, cisapride, or pimozide, warfarin, theophylline, certain benzodiazepines and phenytoin. If fluvoxamine maleate tablets are to be administered together with a drug that is eliminated via oxidative metabolism and has a narrow therapeutic window, plasma levels and/or pharmacodynamic effects of the latter drug should be monitored closely, at least until steady-state conditions are reached (see CONTRAINDICATIONS and WARNINGS).

CNS Active Drugs

Monoamine Oxidase Inhibitors
See WARNINGS

Alprazolam
See WARNINGS

Antipsychotics
See **WARNINGS, Other Potentially Important Drug Interactions and Neuroleptic Malignant Syndrome (NMS) or NMS-Like Reactions.**

Diazepam
See WARNINGS

Alcohol
Studies involving single 40 g doses of ethanol (oral administration in one study and intravenous in the other) and multiple dosing with fluvoxamine maleate (50 mg BID) revealed no effect of either drug on the pharmacokinetics or pharmacodynamics of the other.

Carbamazepine
Elevated carbamazepine levels and symptoms of toxicity have been reported with the co-administration of fluvoxamine maleate and carbamazepine.

Clozapine
Elevated serum levels of clozapine have been reported in patients taking fluvoxamine maleate and clozapine. Since clozapine related seizures and orthostatic hypotension appear to be dose related, the risk of these adverse events may be higher when fluvoxamine and clozapine are co-administered. Patients should be closely monitored when fluvoxamine maleate and clozapine are used concurrently.

Lithium
As with other serotonergic drugs, lithium may enhance the serotonergic effects of fluvoxamine and, therefore, the combination should be used with caution. Seizures have been reported with the co-administration of fluvoxamine maleate and lithium.

Lorazepam
A study of multiple doses of fluvoxamine maleate (50 mg BID) in healthy male volunteers (N=12) and a single dose of lorazepam (4 mg single dose) indicated no significant pharmacokinetic interaction. On average, both lorazepam alone and lorazepam with fluvoxamine produced substantial decrements in cognitive functioning; however, the co-administration of fluvoxamine and lorazepam did not produce larger mean decrements compared to lorazepam alone.

Methadone
Significantly increased methadone (plasma level:dose) ratios have been reported when fluvoxamine maleate was administered to patients receiving maintenance methadone treatment, with symptoms of opioid intoxication in one patient. Opioid withdrawal symptoms were reported following fluvoxamine maleate discontinuation in another patient.

Serotonergic Drugs
Based on the mechanism of action of fluvoxamine and the potential for serotonin syndrome, caution is advised when fluvoxamine is coadministered with other drugs that may affect the serotonergic neurotransmitter systems, such as triptans, linezolid (an antibiotic which is a reversible non-selective MAOI), lithium, tramadol or St. John's Wort (see **WARNINGS, Serotonin Syndrome**).
The concomitant use of fluvoxamine with other SSRIs, SNRIs or tryptophan is not recommended (see **PRECAUTIONS, Drug Interactions**).

Sumatriptan
There have been rare postmarketing reports describing patients with weakness, hyperreflexia, and incoordination following the use of a selective serotonin reuptake inhibitor (SSRI) and sumatriptan. If concomitant treatment with sumatriptan and an SSRI (e.g., fluoxetine, fluvoxamine, paroxetine, sertraline) is clinically warranted, appropriate observation of the patient is advised.

Tacrine
In a study of 13 healthy, male volunteers, a single 40 mg dose of tacrine added to fluvoxamine 100 mg/day administered at steady-state was associated with five- and eight-fold increases in tacrine $C_{max}$ and AUC, respectively, compared to the administration of tacrine alone. Five subjects experienced nausea, vomiting, sweating, and diarrhea following co-administration, consistent with the cholinergic effects of tacrine.

Thioridazine
See CONTRAINDICATIONS and WARNINGS.

Tizanidine
See CONTRAINDICATIONS and WARNINGS.

Tricyclic Antidepressants (TCAs)
Significantly increased plasma TCA levels have been reported with the co-administration of fluvoxamine maleate and amitriptyline, clomipramine or imipramine. Caution is indicated with the co-administration of fluvoxamine maleate tablets and TCAs; plasma TCA concentrations may need to be monitored, and the dose of TCA may need to be reduced.

Tryptophan
Tryptophan may enhance the serotonergic effects of fluvoxamine, and the combination should, therefore, be used with caution. Severe vomiting has been reported with the co-administration of fluvoxamine maleate and tryptophan.

Triptans
There have been rare postmarketing reports of serotonin syndrome with use of an SSRI and a triptan. If concomitant treatment of fluvoxamine with a triptan is clinically warranted, careful observation of the patient is advised, particularly during treatment initiation and dose increases (see **WARNINGS, Serotonin Syndrome**).

**Other Drugs**

Drugs That Interfere With Hemostasis (NSAIDs, Aspirin, Warfarin, etc.)
Serotonin release by platelets plays an important role in hemostasis. Epidemiological studies of the case-control and cohort design that have demonstrated an association between use of psychotropic drugs that interfere with serotonin reuptake and the occurrence of upper gastrointestinal bleeding have also shown that concurrent use of an NSAID or aspirin potentiated the risk of bleeding. Thus, patients should be cautioned about the use of such drugs concurrently with fluvoxamine.

Theophylline
See WARNINGS

Warfarin
See WARNINGS

Alosetron
Because alosetron is metabolized by a variety of hepatic CYP drug metabolizing enzymes, inducers or inhibitors of these enzymes may change the clearance of alosetron. Fluvoxamine is a known potent inhibitor of CYP1A2 and also inhibits CYP3A4, CYP2C9, and CYP2C19. In a pharmacokinetic study, 40 healthy female subjects received fluvoxamine in escalating doses from 50 mg to 200 mg a day for 16 days, with co-administration of alosetron 1 mg on the last day. Fluvoxamine increased mean alosetron plasma concentrations (AUC) approximately 6-fold and prolonged the half-life by approximately 3-fold. (See CONTRAINDICATIONS, **PRECAUTIONS,** and Lotronex™ (alosetron) package insert.)

Digoxin
Administration of fluvoxamine maleate 100 mg daily for 18 days (N=8) did not significantly affect the pharmacokinetics of a 1.25 mg single intravenous dose of digoxin.

Diltiazem
Bradycardia has been reported with the co-administration of fluvoxamine maleate and diltiazem.

Propranolol and Other Beta-Blockers
Co-administration of fluvoxamine maleate 100 mg per day and propranolol 160 mg per day in normal volunteers resulted in a mean five-fold increase (range 2 to 17) in minimum propranolol plasma concentrations. In this study, there was a slight potentiation of the propranolol-induced reduction in heart rate and reduction in the exercise diastolic pressure.

One case of bradycardia and hypotension and a second case of orthostatic hypotension have been reported with the co-administration of fluvoxamine maleate and metoprolol.

If propranolol or metoprolol is co-administered with fluvoxamine maleate tablets a reduction in the initial beta-blocker dose and more cautious dose titration is recommended. No dosage adjustment is required for fluvoxamine maleate tablets.

Co-administration of fluvoxamine maleate 100 mg per day with atenolol 100 mg per day (N=6) did not affect the plasma concentrations of atenolol. Unlike propranolol and metoprolol which undergo hepatic metabolism, atenolol is eliminated primarily by renal excretion.

Effects of Smoking on Fluvoxamine Metabolism
Smokers had a 25% increase in the metabolism of fluvoxamine compared to nonsmokers.

Electroconvulsive Therapy (ECT)
There are no clinical studies establishing the benefits or risks of combined use of ECT and fluvoxamine maleate.

**Carcinogenesis, Mutagenesis, Impairment of Fertility**

Carcinogenesis
There is no evidence of carcinogenicity, mutagenicity or impairment of fertility with fluvoxamine maleate.

There was no evidence of carcinogenicity in rats treated orally with fluvoxamine maleate for 30 months or hamsters treated orally with fluvoxamine maleate for 20 (females) or 26 (males) months. The daily doses in the high dose groups in these studies were increased over the course of the study from a minimum of 160 mg/kg to a maximum of 240 mg/kg in rats, and from a minimum of 135 mg/kg to a maximum of 240 mg/kg in hamsters. The maximum dose of 240 mg/kg is approximately 6 times the maximum human daily dose on a $mg/m^2$ basis.

Mutagenesis
No evidence of mutagenic potential was observed in a mouse micronucleus test, an *in vitro* chromosome aberration test, or the Ames microbial mutagen test with or without metabolic activation.

Impairment of Fertility
In fertility studies of male and female rats, up to 80 mg/kg/day orally of fluvoxamine maleate, (approximately 2 times the maximum human daily dose on a $mg/m^2$ basis) had no effect on mating performance, duration of gestation, or pregnancy rate.

**Pregnancy**

Teratogenic Effects

Pregnancy Category C
In teratology studies in rats and rabbits, daily oral doses of fluvoxamine maleate of up to 80 mg/kg and 40 mg/kg, respectively (approximately 2 times the maximum human daily dose on a $mg/m^2$ basis) caused no fetal malformations. However, in other reproduction studies in which pregnant rats were dosed through weaning there was (1) an increase in pup mortality at birth (seen at 80 mg/kg and above but not at 20 mg/kg), and (2) decreases in postnatal pup weights (seen at 160 mg/kg but not at 80 mg/kg) and survival (seen at all doses; lowest dose tested = 5 mg/kg). (Doses of 5 mg/kg, 20 mg/kg, 80 mg/kg, and 160 mg/kg are approximately 0.1, 0.5, 2, and 4 times the maximum human daily dose on a $mg/m^2$ basis.) While the results of a cross-fostering study implied that at least some of these results likely occurred secondarily to maternal toxicity, the role of a direct drug effect on the fetuses or pups could not be ruled out. There are no adequate and well-controlled studies in pregnant women. Fluvoxamine maleate should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects
Neonates exposed to fluvoxamine maleate and other SSRIs or serotonin and norepinephrine reuptake inhibitors (SNRIs), late in the third trimester have developed complications requiring prolonged hospitalization, respiratory support, and tube feeding. These findings are based on postmarketing reports. Such complications can arise immediately upon delivery. Reported clinical findings have included respiratory distress, cyanosis, apnea, seizures, temperature instability, feeding difficulty, vomiting, hypoglycemia, hypotonia, hypertonia, hyperreflexia, tremor, jitteriness, irritability and constant crying. These features are consistent with either a direct toxic